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(54) Title: LOCAL USE OF IL-1RA IN CORNEAL TRANSPLANT REJECTION OR DISORDERS OF THE EYE

(57) Abstract

Topical application of interleukin-1 receptor antagonist (IL-1ra) is shown to promote corneal transplant survival in a murine model of orthotopic allotransplantation, having a significant effect in prolonging graft survival in both high-risk and normal (low-risk) stromal beds. Furthermore, the promotion of graft survival is associated with a significant decrease in corneal inflammation. Therefore, IL-1ra and related antagonists to interleukin-1 can be used in a therapeutic composition for topical prophylaxis and treatment of allograft rejection and for local treatment of a wide array of immunogenic inflammatory diseases of the eye. The composition comprises a therapeutically effective amount of IL-1ra in association with a pharmaceutically acceptable carrier vehicle for topical application.

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LOCAL USE OF IL-1RA IN CORNEAL TRANSPLANT REJECTION OR DISORDERS OF THE EYE

FIELD OF THE INVENTION

This invention relates to the prophylaxis and treatment of corneal transplant rejection and other immune and inflammatory disorders of the eye and more particularly to a topical treatment therefor.

GOVERNMENT RIGHTS

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BACKGROUND OF THE INVENTION

Corneal transplantation has emerged as the most common and successful form of solid tissue transplantation with over 40,000 cases performed in the United States alone (1). In uncomplicated first allografts performed in avascular beds, the 2-year survival rate is over 90% (2). The extraordinary success of penetrating keratoplasty can be attributed to various features of the normal cornea and anterior segment that in the aggregate account for their "immune-privileged" state (3) including: (a) the avascularity of the stroma, (b) the absence of corneal lymphatics, (c) the rarity of indigenous professional antigen-presenting Langerhans cells (LC) or macrophages in the normal graft bed, (d) a unique spectrum of locally produced immunomodulatory cytokines that suppress immunogenic inflammation and complement activation (to which the cornea itself contributes), and (e) expression

of Fas ligand by these ocular tissues that can directly suppress immunogenic inflammation (4).

In spite of the overall success with corneal transplantation, however, a substantial percentage of corneal grafts experience at least one rejection episode. This is significant since of all the technical and tissue parameters that can affect final graft outcome, immunologic rejection represents the principal threat to allograft longevity regardless of the degree of allodisparity (5,6,7,8,9). This immunologic threat to graft survival is nowhere more evident than in vascularized recipient beds that tend to suffer from earlier and more fulminant rejection episodes that are more resistant to therapy (1,5,7,8,10).

The advent of corticosteroids and their use in the prophylaxis and treatment of corneal transplant rejections has represented the most significant contribution to the prolongation of corneal transplant survival over the last several decades (11,12). However, the local use of corticosteroids, or alternative general immunosuppressants, is associated with significant complications, such as infection, cataracts, glaucoma and corneal thinning (13,14,15,16). General immunosuppressive therapy, when used systemically, may be associated with serious side-effects and multiorgan dysfunction (morbidity) which does at times culminate in death. It is therefore apparent that development of molecular strategies that can specifically target a critical step in the transplant rejection process is desirable and would prove to be an effective modality of circumventing the problems inherent in non-specific immune suppression.

SUMMARY OF THE INVENTION

Interleukin-1 (IL-1) is a potent proinflammatory cytokine that has a wide range of activities including the critical mediation of the acute-phase response, chemotaxis and activation of inflammatory and antigen-presenting cells,

upregulation of adhesion molecules/ costimulatory factors on cells, and stimulation of neovascularization (17,18,19,20). IL-1 has been implicated as an important cytokine in host immunologic reactions to a variety of non-ocular allografts (21,22,23). In the eye, IL-1 activity has been correlated with corneal neovascularization (24), endotoxin-mediated uveitis (25), corneal collagenase and metalloprotease expression (26,27), corneal injury in vitamin-A deficiency (28), and herpetic stromal keratitis (29). Niederkorn and co-workers have shown that IL-1 mediated Langerhans cell migration can play a critical role in host allosensitization in the setting of corneal transplantation (17,30). For all these reasons, IL-1 is an attractive target for therapeutic intervention in immunogenic inflammatory diseases.

Interleukin-1 receptor antagonist (IL-1ra) is a naturally occurring IL-1 isoform with high-affinity binding to both IL-1 receptor subtypes. IL-1ra functions as an active IL-1 inhibitor, having no agonist activity (31,32). There is a 77% homology between the predominant human and murine isoforms of IL-1ra, and systemic administration of recombinant human IL-1ra has been shown to have a profound downregulatory effect on the acute phase cytokine cascade in both man and mouse (33,34).

Others have attempted to assess the potential activity of IL-1ra in inhibiting the immunogenic effects of IL-1, with mixed results. Rosenbaum (57) reports that despite the activity of IL-1ra in inhibiting inflammation induced by the administration of IL-1 intravitreally in a New Zealand white rabbit model for uveitis, IL-1ra did not produce significant reduction in inflammation subsequent to an active Arthus reaction or subsequent to the intravitreal injection of E.coli endotoxin.

Nevertheless, it has surprisingly been found, and is reported here, that direct application of IL-1ra to corneal allografts leads to a significant prolongation of transplant survival. The results described below demonstrate that

IL-1ra administration has a significant positive effect in promoting corneal allograft survival, i.e., in increasing survival rates, of both normal- and high-risk transplant recipients. In addition, both normal- and high-risk IL-1ra treated graft sites had significantly less inflammation and Langerhans cell infiltration compared to untreated controls.

Therefore, the invention is directed to a method for treating allografts and preventing allograft rejection, or for generally treating an immune or inflammatory response of the eye. The method of the invention includes direct, local application of a therapeutic composition to an affected area of a patient. The therapeutic composition useful in the method of the invention comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for local application. Furthermore, the therapeutic composition can be packaged as an article of manufacture of the invention that includes a label indicating the use of the composition in the method of the invention. Preferably, the interleukin-1 antagonist is an interleukin-1 receptor antagonist and, most preferably, the naturally occurring (or recombinant) human IL-1 isoform IL-1ra. Alternatively, other interleukin-1 antagonists may be utilized for the same effect. These include, but are not limited to, (1) modifications of native IL-1ra that would, e.g., render this compound more bioactive, or (2) other IL-1 antagonists that would bind and hence render inactive the IL-1 receptors (e.g., anti-IL-1 receptor antibodies) and/or (3) soluble form(s) of the IL-1 receptor that would bind IL-1 isoforms and prevent their binding to cell-associated receptors. The carrier vehicle in the composition of the invention is preferably a viscous formulation, and most preferably, sodium hyaluronate for application to the corneal surface, to promote a longer residence time for the therapeutic agent at the affected site of the patient. Furthermore, in another method of the invention, sodium hyaluronate (or any other appropriate

hyaluronate salt) can be used as a pharmaceutical carrier vehicle for the delivery of other therapeutic agents to the ocular surface of a patient.

Preferably, the method of the invention is used to prolong transplant survival in corneal allograft recipients. The method of the invention would also be useful for therapeutic intervention in immunogenic inflammatory diseases of the cornea and ocular surface, such as keratoconjunctivitis sicca and other dry eye states including Sjögren's syndrome, allergic conjunctivitis and other atopic conditions of the ocular surface, corneal neovascularization, and immune or infectious keratitis states. In addition, upon local injection or irrigation, the method of the invention would be useful for suppressing diseases such as uveitis and post-surgical inflammation in intraocular compartments (e.g., anterior chamber or vitreous cavity). Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a conceptual relationship among corneal neovascularization, IL-1ra treatment, and graft outcome based on degree of preoperative risk;

Fig. 2 shows Kaplan-Meier survival curves for normal-risk and high-risk corneal allograft recipients;

Figs. 3A and 3B show association between corneal allograft survival and neovascularization score in normal-risk recipients based on IL-1ra treatment; and

Fig. 4A and 4B show association between corneal allograft survival and neovascularization score in high-risk recipients based on IL-1ra treatment.

DETAILED DESCRIPTION OF THE INVENTION

The currently available pharmaceutic armamentarium for corneal transplant survival is primarily composed of

corticosteroids. Their introduction into ophthalmology is arguably the single most significant factor in the last four decades' advances in corneal transplant surgery (13). Nevertheless, beyond their well-known serious complications, corticosteroids show widely variable efficacy in preventing ultimate immunogenic graft failure, and this is particularly the case in high-risk keratoplasty (1,7). This series of experiments was conducted to test whether the specific inhibition of the important proinflammatory cytokine IL-1, by application of IL-1 receptor antagonist (IL-1ra), could be successful in prolonging either normal- or high-risk orthotopic corneal allografts in the mouse.

For all experiments, C57BL/6 corneas were transplanted into BALB/c (major histocompatibility [MHC] and minor H-disparate) eyes. "High-risk" transplants consisted of transplants that were sutured into BALB/c recipient beds with corneal neovascularization induced by placement of three interrupted sutures in the host cornea two weeks previously. Both risk groups were divided in a masked fashion into treatment subgroups that received either 20mg/ml of IL-1ra mixed in 0.2% sodium hyaluronate vehicle (N=28) or placebo alone (N=25). All transplants were evaluated for 8 weeks postoperatively for signs of rejection. Any changes in the degree of corneal neovascularization were also determined. At the end of follow-up, corneal specimens were processed for enumeration of Langerhans cells and for histopathological evaluation.

The results show a significant increase in the survival rates of both normal- and high-risk transplants among the IL-1ra-treated animals compared to untreated controls by both stratified Mantel-Haenszel (P=0.02) and Kaplan-Meier survival (P=0.03) analyses. Furthermore, both normal- and high-risk IL-1ra treated grafts have significantly less inflammation and Langerhans cells infiltration compared to untreated controls.

There is little doubt that presence of corneal neovascularization is a significant risk factor for corneal allograft survival (1,6,7,10,36). Therefore, the relationship between IL-1ra treatment and neovascularization scores was also examined. In the normal-risk, but not in the high-risk setting where neovascularization had been induced two weeks previously, IL-1ra treatment was determined to be associated with a blunted postkeratoplasty neovascularization response. Laboratory results show that IL-1ra can significantly blunt the early, but not late, phase corneal neovascularization development in response to standard angiogenic stimuli, suggesting that there are non-IL-1 mediated factors that can overshadow IL-1 suppression in corneal angiogenesis. The failure of IL-1ra to lead to significant neovascularization regression in the high-risk beds, as opposed to its capacity for angiostasis in the normal-risk beds as demonstrated here, is apparently due to the dominance of non-IL-1 driven angiogenic factors in the former. However, IL-1ra appears to play an important role, possibly in combination with other agent(s) in suppressing the neovascular response.

In the aggregate, development of corneal neovascularization causes sufficient perturbation of the ocular microenvironment to lead to a loss of "immune privilege" as measured by the ability to induce anterior chamber-associated immune deviation (ACAID) (35). However, in contrast to the expectation that the efficacy with which IL-1ra could blunt rejection in the high-risk corneas would be paralleled by an equal degree of suppression in corneal neovascularization in the high-risk setting, it was determined that treatment with IL-1ra promotes allograft survival in recipients regardless of their neovascularization status. This effect could mirror what has been described previously in neovascularized corneas where therapeutic measures that have been shown to restore immune

privilege/ACAID are associated with highly variable degrees of angiostasis (35).

From the results described above, it appears that an interrelationship exists among corneal neovascularization, IL-1ra treatment and graft outcome based on the degree of preoperative risk. Referring to Fig. 1, in normal-risk (or virgin) corneal beds **10**, suppression of IL-1 activity by IL-1ra treatment **12** has a significant dampening effect on corneal angiogenesis, reducing neovascularization to a mild (O/++NV) or minimal (0/+NV) degree **14**. Furthermore, treatment with IL-1ra **12** has a significant effect on promoting graft longevity **16**. In the neovascularized cornea **18**, the effect of IL-1ra treatment **20** on corneal transplantation **16** appears to be independent of the degree of corneal neovascularization, which remains at the significant (+++NV) level **22**. Specifically, IL-1ra treatment of high-risk beds (with antecedent neovascularization) does not appear to significantly suppress neovascularization; however, this same treatment leads to significant prolongation of graft survival compared to untreated controls.

The degree to which the migration of Langerhans cells (LC) into the central cornea can be blunted by application of IL-1ra was intriguing. Since the healthy and unoperated cornea is essentially devoid of these constitutively antigen-presenting cells as well as other MHC class II-bearing "passenger leukocytes," the presence of LC in the central cornea has been implicated in the loss of local immune privilege, by virtue of their critical role in immune surveillance and allosensitization in the "indirect pathway" (2,17,36). IL-1 has been shown to be a critical regulator of LC migration in the cornea (17), and the activity of epidermal LC is known to be at least partially controlled by IL-1 (30,49). Hence, the demonstrated constitutive expression by normal corneal cells of IL-1ra (50) likely plays an important immune regulatory role in the

avascular/non-traumatized cornea by keeping the microenvironment in an inhospitable site for sensitization. The results reported here, showing a decrease in LC numbers in IL-1ra treated corneas compared to untreated controls, are evidence that in traumatized corneas the induction of allosensitization in the corneal allograft can be tilted in favor of unresponsiveness by the application of high-doses of IL-1ra. This effect is reflected in greater longevity of these allografts.

The specific regimen utilized in these studies for the delivery of IL-1ra to the cornea was based on the observation that ocular bioavailability of topical medications is enhanced in viscous formulations (51,52). Traditional formulations that rely on aqueous drops for topical treatment often provide low bioavailability because of efficient elimination processes active on the ocular surface which typically lead to a very short drug residence time. The choice of sodium hyaluronate (SH) as the preferred vehicle in this series of experiments was based on previous observations that 0.2% SH has a very long contact (residence) time on the ocular surface. This vehicle is also well-tolerated due to its pseudoplastic biophysical properties that offer little resistance to high shear rates (52,53).

The following examples are presented to illustrate the advantages of the present invention and to assist one of ordinary skill in making and using the same. These examples are not intended in any way otherwise to limit the scope of the disclosure.

EXAMPLE I

Corneal Transplant Survival Following IL-1ra Treatment

Fifty-five corneal allografts were performed in 55 BALB/c mice, of which 53 were deemed technically acceptable for long-term follow-up; that is, anterior segment integrity was maintained with no signs of wound leak, infection, or

hyphema. These were subdivided, based on degree of immunologic risk as described above, into normal- (N=28) and high-risk (N=25) groups.

Topical preparations of the therapeutic agent were applied to the recipient mice three times daily for the 56 days (8 weeks) duration of the study. The study medication was composed of 20 mg/ml of human recombinant IL-1ra in 0.2% sodium hyaluronate in PBS (supplied by Amgen, Boulder, CO). Placebo-treated animals received the vehicle 0.2% sodium hyaluronate only. Graft success or failure was established based on opacity scores, as detailed in Materials and Methods below.

Statistical analysis of cumulative rejection rates, after stratification for degree of risk based on recipient bed vascularity, revealed a strong association between IL-1ra treatment and graft survival (Mantel-Haenszel test, P=0.02). The 8-week incidences of transplant rejection were lowest in the normal-risk grafts that were treated with IL-1ra (7%), and highest in the high-risk grafts that received vehicle only (73%) (Table I).

Table I

Corneal transplant rejection rates, stratified by IL-1a therapy and degree of risk.

Degree of Risk	N	Rejection rate*	Rejection Reaction rate$^{\Psi}$
Normal Risk			
treated	14	7%	7%
untreated	14	29%	50%
High Risk↑			
treated	14	36%	43%
untreated	11	73%	91%
Total			
treated	28	21%	25%
untreated	25	48%	68%

* Cumulative rejection rate over 8-week follow-up period.

Ψ Includes opacification score of ≥ 2+ at any time point after 2 weeks, as described in Materials and Methods.

↑ High-risk transplants are by definition grafted into vascularized stromal beds, as described in Materials and Methods.

Four of the animals used developed dystrophic/degenerative corneal calcific deposits following surgery. Because of the reported association between this common BALB/c corneal finding and loss of immune privilege in the anterior segment (39), these animals were censored from further evaluation prior to completion of the 8-week follow-up course. To prevent generation of bias by artificially lowering the denominator size in 8-week rate calculations, survival curves were developed. Referring to Fig. 2, Kaplan-Meier survival curves for normal-risk (N=28) and high-risk (N=25) corneal allograft recipients are shown, stratified by treatment with IL-1ra active agent or placebo. Survival analysis revealed that IL-1ra treatment was associated with significant graft longevity in both normal-risk (P=0.1) and high-risk (P<0.05) recipient beds, with an overall reduction in rejection rate of 56% (P=0.03). Thus, in both cases there is an association between IL-1ra treatment and transplant outcome, as survival rates of high-risk grafts treated with the active agent closely mirror those of normal-risk transplants receiving placebo.

EXAMPLE II

Corneal Neovascularization

In addition to graft survival/opacification criteria detailed in Materials and Methods, transplants were also followed biomicroscopically for the degree of corneal neovascularization. All high-risk beds had been specially prepared for two weeks to develop two or more quadrants of stromal neovascularization as described previously (36), and all normal-risk corneal beds were avascular.

It has been shown previously, in both man (40) and mouse (37), that corneal transplantation alone can induce neovascularization. Since post-keratoplasty corneal neovascularization likely plays an important role in facilitating effector elements in the inflamed cornea (35,40), the corneas were also examined to see if treatment

with IL-1ra had an appreciable effect on this parameter, and an angiostatic effect with IL-1ra treatment in the normal-risk, but not high-risk, transplants was observed.

Referring to Figs. 3A and 3B, among the normal-risk grafts, 38% of the untreated corneas (Fig. 3A) had a neovascularization score of ≥3 at 4 weeks compared to none of the IL-1ra treated cases (Fig. 3B). Respective rates at 8 weeks were 31% for untreated controls and 18% for treated cases. In contrast, referring to Figs. 4A and 4B, no significant association of angiostatic effect with IL-1ra treatment was apparent in high-risk eyes that had been induced to have corneal neovascularization two weeks previously. The proportions of corneas with a neovascularization score of ≥3 at 4 and 8 weeks follow-up was very comparable between untreated controls (Fig. 4A) (91% at both time points) and treated cases (Fig. 4B) (100% and 86% at respective time points).

Furthermore, there was a significant correlation between corneal angiogenesis and rejection in both untreated normal- and high-risk controls. Among untreated normal-risk transplants (Fig. 3A), 4 of 4 corneas with a neovascularization score of ≥3 had rejected at 8 weeks. Similarly, among untreated high-risk recipients (Fig. 4A), 7 of 10 grafts with a neovascularization score of ≥3 had rejected at 4 weeks, and 8 of 10 grafts with neovascularization ≥3 had rejected at 8 weeks follow-up. In contrast, there was a distinct divergence between corneal angiogenesis and graft survival among both normal- and high-risk transplants treated with IL-1ra. For example, among the normal-risk transplants that had received treatment with IL-1ra (Fig. 3B), the one allograft that rejected at 8 weeks had minimal neovascularization, and two treated grafts with significant neovascularization never rejected. Similarly, among the treated high-risk transplants (Fig. 4B), 5/12 grafts with significant neovascularization had rejected at

8 weeks, and almost the same proportion (7/12) had not rejected.

EXAMPLE III

Langerhans Cell Population.

The presence of Langerhans cells (LC) in the cornea has been associated with immunogenic inflammation, the host's ability to be allosensitized, and loss of immune privilege (39,41,42). To explore this point further, naive age-matched BALB/c corneas and allografts were excised at the completion of the follow-up period to assay their LC populations with fluorescence microscopy, as described in Materials and Methods.

Consistent with previous findings, the central and paracentral areas of normal naive and avascular corneas had very few LC. Allogeneic transplants led to a significant increase in the number of LC in the central portions of the cornea. Interestingly, however, treatment with IL-1ra had a significant dampening effect on LC migration, regardless of the degree of pre- or postoperative corneal neovascularization. Among the normal-risk allografts, the average number of central corneal LC in the IL-1ra treated corneas was $13/mm^2$ compared to $41/mm^2$ (32%) in the untreated controls (P=0.03). A similar reduction in the number of LC was observed after IL-1ra treatment in the vascularized high-risk recipient beds where the number of LC was $27/mm^2$ in the treated corneas compared to $89/mm^2$ in the untreated eyes (P=0.02).

EXAMPLE IV

Corneal Inflammation

Histopathological evaluation of IL-1ra treated and untreated corneas, at 8 weeks, in both normal- and high-risk eyes demonstrated a noticeable decrease in the number of infiltrating leukocytes (particularly neutrophils) into grafts that had undergone treatment, with an associated decrease in the degree of stromal edema. In spite of

comparable degrees of clinically evident corneal neovascularization in the untreated and treated high-risk grafts, as described above, there was still an appreciable difference in the level of neutrophil infiltration between treated corneas and vehicle-treated controls. The decreased corneal inflammation in the IL-1ra-treated allografts was reflected by a generally lower opacity score (irrespective of final rejection status) in the IL-1ra-treated transplants. For example, all but one of the untreated normal-risk grafts that eventually failed developed opacity scores ≥3; whereas the single IL-1ra treated normal-risk graft that failed had an opacity score of 2.

Materials and Methods

Mice and anesthesia. Six to ten-week old BALB/c (H-2^d) and C57BL/6 (H-2^b) mice were purchased (Taconic, New York) or obtained from the Schepens Eye Research Institute animal colony. All animals were treated according to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Each animal was deeply anesthetized with an intramuscular injection of 3 to 4 mg ketamine and 0.1 mg xylazine prior to all surgical procedures.

Corneal transplantation. All 55 transplants involved combined MHC- and minor alloantigen disparate corneas that were grafted from C57BL/6 donors into BALB/c eyes as follows. On day 0, in each case, the central 2-mm of the donor cornea was marked with a microcurrette and the donor button excised by Vannas scissors and placed in phosphate-buffered saline (PBS). The recipient graft bed was prepared by excising the central 2-mm of the cornea. The donor button was then secured in place with 8 interrupted 11-0 nylon sutures (Sharppoint; Vanguard, Houston, TX). Antibiotic ointment was applied to the corneal surface and the lids were shut for 24 hours with an 8-0 nylon tarsorrhaphy for the next day after

which treatment would start. Animals were divided in a masked fashion into cases that would receive active IL-1ra and controls that would receive vehicle/placebo alone as detailed below. Grafted eyes with technical difficulties (hyphema, infection, or loss of anterior chamber) were excluded from study. Transplant sutures were removed in all cases on day 7.

Induction and grading of corneal neovascularization. Intrastromal sutures induce robust neovascularization growth into the normally avascular corneal stroma from the limbus that can be appreciated as early as three days following suture placement (35), and untreated allografts into these high-risk beds are rejected swiftly (36). Two parallel protocols were devised to study normal- and high-risk corneal transplantation. In the former case, animals were left unmanipulated until the day of surgery. High-risk beds were developed as described previously (36). Briefly, three interrupted 11-0 sutures were placed in the central cornea of one eye of a normal BALB/c mouse on day -14 under aseptic microsurgical technique using an operating microscope. The neovascularized beds then served as high-risk graft beds for orthotopic corneal transplants on day 0 as described above (neovascularization-inducing sutures were removed at the time of transplantation). Neovascularization was graded between 0-8 as described previously based on the degree of centripetal ingrowth and quandrantic involvement of the neovessels (37).

Evaluation and scoring of orthotopic corneal transplants. Grafts were evaluated by slitlamp biomicroscopy twice a week. At each time point grafts were scored for opacification. A previously described scoring system (37) was used to measure the degree of opacification between 0-5+: 0=clear and compact graft; 1+=minimal superficial opacity; 2+=mild deep (stromal) opacity with pupil margin and iris

vessels visible; 3+=moderate stromal opacity with only pupil margin visible; 4+=intense stromal opacity with the anterior chamber visible; 5+=maximal corneal opacity with total obscuration of the anterior chamber. Grafts with an opacity score of 2+ or greater after three weeks were considered as rejected (immunologic failure); grafts with an opacity score of 3+ or greater at two weeks that never cleared were also regarded as rejected. Since some grafts had only transient opacification, grafts with an opacity score of 2+ or greater at any time point after two weeks were considered to have a rejection reaction (RR), regardless of the opacity score at eight weeks (37).

Langerhans cell (LC) enumeration and histopathological evaluation. The LC were assessed by an immunofluorescence assay performed on whole corneal epithelial mounts as previously described (38). Briefly, each eye was enucleated and the anterior segment dissected under the operating microscope. The cornea was placed in 20mM ethylenediaminetetraacetic acid (EDTA) buffer and incubated for 30 minutes at 37°C, followed by removal of the epithelium in toto and washed in PBS at room temperature. The cornea was then fixed with 95% ethanol prior to washing and incubation with 1:20 diluted primary anti-murine Ia^d antibody for 45 minutes at 37°C. The tissue was then washed in PBS and incubated with a FITC-labeled goat anti-mouse secondary antibody for 30 minutes at 37°C. Negative controls either bypassed this step or were incubated with antibody specific for an unrelated MHC epitope. Sections were then mounted on slides and examined under the fluorescent microscope with a square ocular grid where LC were enumerated. Corneal specimens that were not processed for LC enumeration were fixed, sectioned, and stained with hematoxylin-eosin for light microscopic evaluation.

<u>Statistical techniques</u>. The proportional rates of rejected allografts in the IL-1ra and vehicle-only treated groups were compared using two methods. First, the Mantel-Haenszel summary chi-square statistic was obtained, stratified by (adjusted for) degree of preoperative risk (i.e., normal vs. vascularized stromal bed) to compare the proportion of rejected transplants in the two groups. Second, Kaplan-Meier survival curves were constructed in order to compare the probability of graft survival over the follow-up period, both overall and separately for normal- and high-risk eyes, in the IL-1ra treated and untreated controls. This method accounted for the variability in the time-to-graft rejection in addition to the variation in follow-up time (4 mice in the normal-risk group had follow-up terminated prior to the end of the 8-week period). Comparison of Langerhans cell population means among IL-1ra treated eyes and untreated controls was made by the Student's t-test.

<u>Use</u>

IL-1ra is a very promising agent for use in corneal transplantation, both because of its efficacy as demonstrated in these experimental results and its putative value over existing therapy, which has well-known side-effects and complications. In addition, the very significant dampening of the inflammatory response observed suggests that treatment with IL-1ra and other antagonists of IL-1 and its receptors can be applied to a wide variety of ocular immune and inflammatory disorders.

IL-1 antagonism, e.g., via use of IL-1ra, can suppress immunogenic inflammation, as demonstrated in the corneal transplant model herein, in both virgin and previously inflamed/neovascularized eyes. In the eye, the topical administration of IL-1ra can include non-transplant therapeutic uses such as treatment of allergic and hypersensitivity disorders of the ocular surface, burns,

infections, dry eye disorders, and chronic inflammatory states that may lead to neovascularization and/or scarring or fibrosis of the cornea and ocular surface. In addition to sodium hyaluronate, other vehicles, e.g., cyclodextrins may be used to increase drug deliver to the surface epithelium.

The ocular use of IL-1ra is not limited to topical administration to the cornea and ocular surface. Intraocular administration, e.g., by intraocular injection into the anterior chamber or irrigation at the time of surgery, is appropriate for treatment (or prophylaxis of recurrence) of intraocular inflammatory disorders such as autoimmune or infectious uveitis, post-traumatic or post-surgical inflammation, or idiopathic uveitides. Sustained release formulations, e.g., with use of biodegradable or non-degradable biocompatible polymers, or simple irrigation of these agent(s) at the time of surgery, can be used for intraocular delivery of IL-1ra to subjects.

Other candidate interleukin-1 antagonists that might be useful in the methods of the invention, as described earlier, can be tested for effectiveness using one of the assays described herein (e.g., measuring the extent of corneal inflammation, neovascularization, graft survival or Langerhans cell migration) and the results compared to those obtained with IL-1ra.

The dosage of IL-1ra used in the experiments described herein was relatively high in order to determine the maximum positive effect of treatment. However, IL-1ra appears to be able to exert its suppressive effect over a wide dose range (56). Optimal dosage and appropriate modes of administration for each of the conditions delineated above can be determined by conventional protocols. For example, in the case of corneal transplantation, other doses ranging between 20ng/ml - 2mg/ml will additionally be tested and the endpoints described above (e.g., effect on corneal inflammation, neovascularization, graft longevity or Langerhans cell

migration) for the tested dosage will be compared to those obtained using the current dose herein of 20mg/ml. It is to be expected that an appropriate concentration of an IL-1 receptor antagonist in a vehicle for local administration to a human patient will be in the range of 20ng/ml to 20 mg/ml.

References:

1. The Collaborative Corneal Transplantation Studies Research Group, The collaborative corneal transplantation studies (CCTS); "Effectiveness of histocompatibility matching in high-risk corneal transplantation," Arch. Ophthalmol. 110:1392 (1992).

2. Niederkorn, "Immune privilege and immune regulation in the eye," Adv. Immunol. 48:191 (1990).

3. Streilein, "Immunological non-responsiveness and acquisition of tolerance in relation to immune privilege in the eye," Eye 9:236 (1995).

4. Griffith et al., "Fas ligand-induced apoptosis as a mechanism of immune privilege," Science 270:1189 (1995).

5. Mader et al., "The high-risk penetrating keratoplasty," Ophthalmol Clin. North Am. 4:411 (1991).

6. Coster, "Mechanisms of corneal graft failure: the erosion of corneal privilege," Eye 2:251 (1989).

7. Maguire et al, "Risk factors for corneal graft failure and rejection in the collaborative corneal transplantation studies," Ophthalmology 101:1536 (1994).

8. Williams et al., "Factors predictive of corneal graft survival; Report from the Australian Corneal Graft Registry, Ophthalmology 99:403 (1992).

9. Alldredge et al., "Clinical types of corneal transplant rejection. Their manifestations, frequency, preoperative correlates, and treatment," Arch. Ophthalmol. 99:599 (1981).

10. Volker et al., "Hierarchy of prognostic factors for corneal allograft survival," Austr. NZ J. Ophthalmol. 15:11 (1987).

11. Wilson et al., "Graft failure after penetrating keratoplasty," Surv. Ophthalmol. 34:325 (1990).

12. Hill et al., "Corticosteroids in corneal graft rejection. Oral versus single pulse therapy," Ophthalmology 98:329 (1991).

13. Raizman, "Corticosteroid therapy of eye disease. Fifty years later," Arch. Ophthalmol. 114:1000 (1996).

14. Hemady et al., "Immunosuppressive drugs in immune and inflammatory ocular disease," Surv. Ophthalmol. 35:369 (1991).

15. Barraquer, "Immunosuppressive agents in penetrating keratoplasty," Am. J. Ophthalmol. 100:61 (1985).

16. Frangie et al., "Steroids," Int. Ophthalmol. Clin. 33:9 (1993).

17. Niederdorn et al., "Phagocytosis of particulate antigens by corneal epithelial cells stimulates interleukin-1 secretion and migration of Langerhans cells into the central cornea," Reg. Immunol. 2:83 (1989).

18. Dinarello et al., "The role of interleukin-1 in disease," New Eng. J. Med. 328:106 (1993).

19. Le et al., "Tumor necrosis factor and interleukin 1: cytokines with multiple overlapping biological activities," Lab. Invest. 56:234 (1987).

20. De Vos et al., "Cytokines and uveitis, a review," Curr. Eye Res. 11:581 (1992).

21. Buchwald et al., "Clinical value of interleukin 1- and interleukin 2-determinations in patients after kidney transplantation," Allergie und Immunologie 36:137 (1990).

22. Takasu et al., "A new immunosuppressant, 15-deoxyspergualin, inhibits production of IL-1 from isolated hepatic sinusoidal lining cells in swine liver transplantation," Transplant Proc. 21:1081 (1989).

23. Tilg et al., "Evaluation of cytokines and cytokine-induced secondary messages in sera of patients after liver transplantation," Transplantation 49:1074 (1990).

24. BenEzra et al., "In vivo angiogenic activity of interleukins," Arch. Ophthalmol. 108:573 (1990).

25. Kijlstra, "The role of cytokines in ocular inflammation," Br. J. Ophthalmol. 78:885 (1994).

26. Girard et al., "Transforming growth factor-beta and interleukin-1 modulate metalloproteinase expression by corneal stromal cells," Invest. Ophthalmol. Vis. Sci. 32:2441 (1991).

27. West-Mays et al., "Competence for collagenase gene expression by tissue fibroblasts requires activation of an interleukin 1 alpha autocrine loop," Proc. Natl. Acad. Sci. USA 92:6768 (1995).

28. Shams et al., "Increased interleukin-1 activity in the injured vitamin A-deficient cornea," Cornea 13:156 (1994).

29. Staats et al. "Cytokine expression in vivo during murine herpetic stromal keratitis. Effect of protective antibody therapy," J. Immunol. 151:277 (1993).

30. Niederkorn, "Effect of cytokine-induced migration of Langerhans cells on corneal allograft survival," Eye 9:215 (1995).

31. Hannum et al., "Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor," Nature 343:336 (1990).

32. Eisenberg et al., "Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist," Nature 343:341 (1990).

33. Antin et al., "Recombinant human interleukin-1 receptor antagonist in the treatment of steroid-resistant graft-versus-host disease," Blood 84:1342 (1994).

34. Ohlsson et al., "Interleukin-1 receptor antagonist reduces mortality from endotoxin shock," Nature 348:550 (1990).

35. Dana et al., "Loss and restoration of immune privilege in eyes with corneal neovascularization," Invest. Ophthalmol. Vis. Sci. 37:in press (1996).

36. Sano et al., "Fate of orthotopic corneal allografts in eyes that cannot support anterior chamber-associated immune deviation induction," Invest. Ophthalmol. Vis. Sci. 36:2176 (1995).

37. Sonoda et al. "Orthotopic corneal transplantation in mice--evidence that the immunogenetic rules of rejection do not apply," Transplantation 54:694 (1992).

38. Gillette et al., "Langerhans cells of the ocular surface," Ophthalmology 89:700 (1982).

39. Williamson et al., "Immunobiology of Langerhans cells on the ocular surface. I. Langerhans cells within the central cornea interfere with induction of anterior chamber associated immune deviation," Invest. Ophthalmol. Vis. Sci. 28:1527 (1987).

40. Dana et al., "Corneal neovascularization after penetrating keratoplasty," Cornea 14:604 (1995).

41. McLeish et al., "Immunobiology of Langerhans cells on the ocular surface. II. Role of central corneal Langerhans cells in stromal keratitis following experimental HSV-1 infection in mice," Reg. Immunol. 2:236 (1989).

42. Van der Veen et al., "Prevention of corneal allograft rejection in rats treated with subconjunctival injections of liposomes containing dichloromethylene diphosphonate," Invest. Ophthalmol. Vis. Sci. 35:3505 (1994).

43. Streilein et al., "Immunosuppressive properties of tissues obtained from eyes with experimentally manipulated corneas," Invest. Ophthalmol. Vis. Sci. 37:413 (1996).

44. Briscoe et al., "Antigen-dependent activation of T helper cell subsets by endothelium," Transplantation 59:1638 (1995).

45. Pober et al., "Immunologic interactions of T lymphocytes with vascular endothelium," Adv. Immunol. 50:261 (1991).

46. Collin, "Corneal lymphatics in alloxan vascularized rabbit eyes," Invest. Ophthalmol. 5:1 (1966).

47. Pabilack et al., "Differential expression of human corneal and perilimbal ICAM-1 by inflammatory cytokines," Invest. Ophthalmol. Vis. Sci. 33:564 (1992).

48. Gerritsen et al., "Cytokine activation of human macro- and microvessel-derived endothelial cells," Blood Cells 19:325 (1993).

49. Heufler et al., "Granulocyte/macrophage colony-stimulating factor and interleukin 1 mediate the maturation of murine epidermal Langerhans cells into potent immunostimulatory dendritic cells," J. Exp. Med. 167:700 (1988).

50. Kennedy et al., "Novel production of interleukin-1 receptor antagonist peptides in normal human cornea," J. Cl. Invest. 95:82 (1995).

51. Burstein, "Basic science of ocular pharmacology," In: Bartlett JD, Jaanus SD, eds. Clinical Ocular Pharmacology, 2nd ed. Boston: Butterworth 3 (1989).

52. Saettone et al., "The effect of different ophthalmic vehicles on the activity of tropicamide in man," J. Pharm. Pharmacol. 32:519 (1980).

53. Snibson et al., "Ocular surface residence times of artificial tear solution," Cornea 11:288 (1992).

54. Sand et al., "Sodium hyaluronate in the treatment of keratoconjunctivitis sicca. A double masked clinical trial," Acta. Ophthalmol. 67:181 (1989).

55. Shams et al., "Interferon-gamma, Staphylococcus aureaus, and lipopolysaccharide/silica enhance interleukin-1 beta

production by human corneal cells," Reg. Immunol. 2:136 (1989).

56. Kondo et al., "Interleukin-1 receptor antagonist suppresses contact hypersensitivity," J. Invest. Dermatol. 105:334 (1995).

57. Rosenbaum et al., "Activity of an interleukin 1 receptor antagonist in rabbit models of uveitis," Arch. Ophthalmol. 110:547 (1992).

58. Dinarello, "Interleukin-1 and interleukin-1 antagonism," Blood 77:1627 (1991).

While the present invention has been described in conjunction with a preferred embodiment, one of ordinary skill, after reading the foregoing specification, will be able to effect various changes, substitutions of equivalents, and other alterations to the compositions and methods set forth herein. It is therefore intended that the protection granted by Letters Patent hereon be limited only by the definitions contained in the appended claims and equivalents thereof.

CLAIMS

What is claimed is:

1. A method for prophylaxis or treatment of corneal transplant rejection comprising

providing a corneal transplant recipient patient; and

topically applying a therapeutic composition to an affected area of said patient, wherein said therapeutic composition comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for topical application.

2. A method for prophylaxis or treatment of an immunogenic inflammatory disease comprising

providing a patient suffering from or believed to be at risk from an immunogenic inflammatory disease of the eye; and

topically applying a therapeutic composition to an affected area of said patient, wherein said therapeutic composition comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for topical application.

3. A method for prophylaxis or treatment of an immunogenic inflammatory disease in an intraocular compartment comprising

providing a patient suffering from or believed to be at risk from an immunogenic inflammatory disease in an intraocular compartment; and

locally applying a therapeutic composition to an affected area of said intraocular compartment of said patient, wherein said therapeutic composition comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for local application.

4. A method for prophylaxis or treatment of corneal neovascularization comprising

providing a patient suffering from or believed to be at risk from corneal neovascularization; and

topically applying a therapeutic composition to an affected area of said patient, wherein said therapeutic composition comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for topical application.

5. The method of claim 2 wherein said patient is suffering from keratoconjunctivitis sicca, allergic conjunctivitis, corneal neovascularization, or immune or infectious keratitis states.

6. The method of claim 3 wherein said patient is suffering from uveitis or post-surgical inflammation.

7. The method of claim 3 wherein said applying step is by intraocular injection into the anterior chamber.

8. The method of claim 3 wherein said applying step is by intraocular irrigation at the time of surgery.

9. The method of claim 1, claim 2, claim 3 or claim 4 wherein said interleukin-1 antagonist in said therapeutic composition is an interleukin-1 receptor antagonist.

10. The method of claim 1, claim 2, claim 3 or claim 4 wherein said interleukin-1 antagonist in said therapeutic composition is IL-1ra.

11. The method of claim 1, claim 2, claim 3 or claim 4 wherein said carrier vehicle in said therapeutic composition comprises sodium hyaluronate.

12. A method for prophylaxis or treatment of corneal transplant rejection comprising

providing a corneal transplant recipient patient; and

topically applying a therapeutic composition to an affected area of said patient, wherein said therapeutic composition comprises a therapeutically effective amount of IL-1ra in association with a pharmaceutically acceptable carrier vehicle for topical application, said vehicle comprising sodium hyaluronate.

13. An article of manufacture comprising packaging material and a therapeutic composition contained within said packaging material, wherein the therapeutic composition is therapeutically effective for prophylaxis or treatment of corneal transplant rejection and wherein the packaging material comprises a label that indicates that the therapeutic composition can be used topically for prophylaxis or treatment of corneal transplant rejection, and

wherein said therapeutic composition comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for topical application.

14. An article of manufacture comprising packaging material and a therapeutic composition contained within said packaging material, wherein the therapeutic composition is therapeutically effective for prophylaxis or treatment of an immunogenic inflammatory disease of the eye and wherein the packaging material comprises a label that indicates that the therapeutic composition can be used topically for prophylaxis or treatment of an immunogenic inflammatory disease of the eye, and

wherein said therapeutic composition comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for topical application.

15. An article of manufacture comprising packaging material and a therapeutic composition contained within said packaging material, wherein the therapeutic composition is therapeutically effective for prophylaxis or treatment of an immunogenic inflammatory disease in an intraocular compartment and wherein the packaging material comprises a label that indicates that the therapeutic composition can be used locally for prophylaxis or treatment of an immunogenic inflammatory disease in an intraocular compartment of a patient, and

wherein said therapeutic composition comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for local application.

16. An article of manufacture comprising packaging material and a therapeutic composition contained within said packaging material, wherein the therapeutic composition is therapeutically effective for prophylaxis or treatment of corneal neovascularization and wherein the packaging material comprises a label that indicates that the therapeutic composition can be used topically for prophylaxis or treatment of corneal neovascularization, and

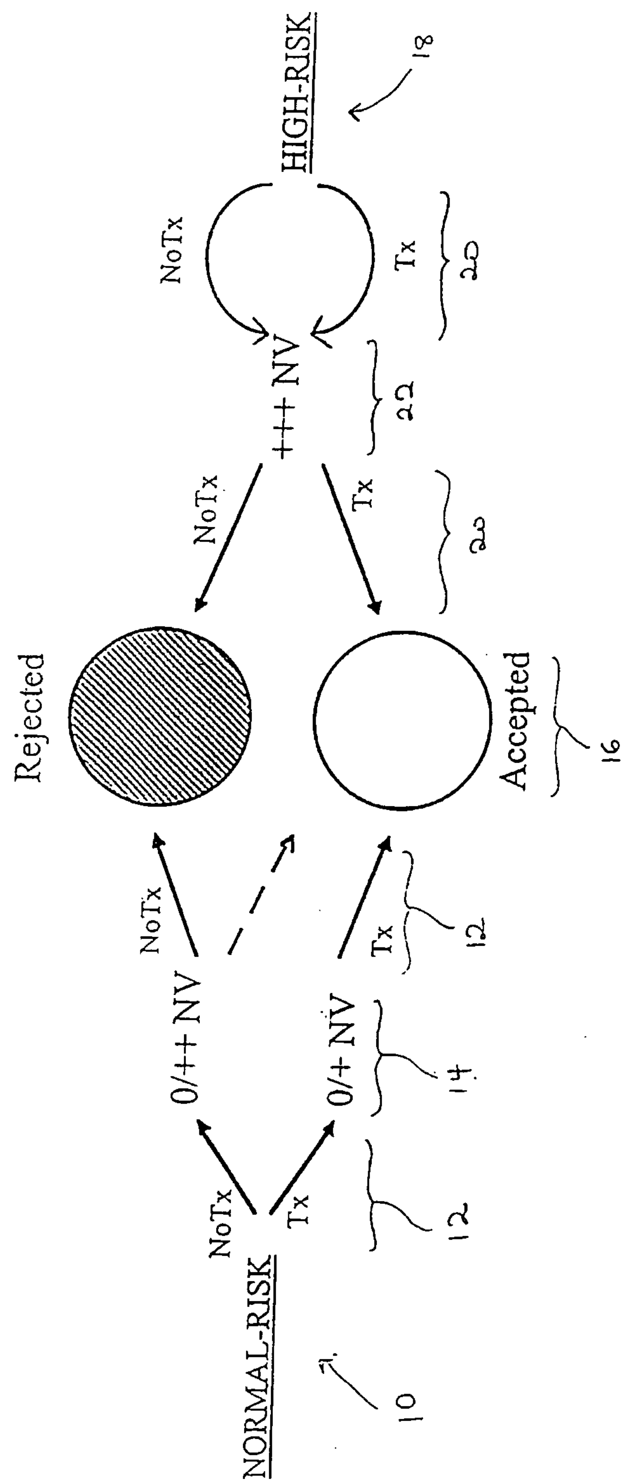
wherein said therapeutic composition comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for topical application.

17. The article of manufacture of claim 13, claim 14, claim 15 or claim 16 wherein, in said therapeutic composition, said interleukin-1 antagonist is an interleukin-1 receptor antagonist.

18. The article of manufacture of claim 13, claim 14, claim 15 or claim 16 wherein, in said therapeutic composition, said interleukin-1 antagonist is IL-1ra.

19. The article of manufacture of claim 13, claim 14, claim 15 or claim 16 wherein, in said therapeutic composition, said carrier vehicle comprises sodium hyaluronate.

20. A method for administering a therapeutic agent to an ocular surface of an eye of a patient comprising topically applying a therapeutic composition to the ocular surface of said patient, wherein said therapeutic composition comprises a therapeutically effective amount of a therapeutic agent in association with a pharmaceutically acceptable carrier vehicle for topical application, said carrier vehicle comprising a hyaluronate salt.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18
NoTx
Tx
20
+++ NV
22
NoTx
Tx
20
Rejected
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NORMAL-RISK
10

Fig. 1

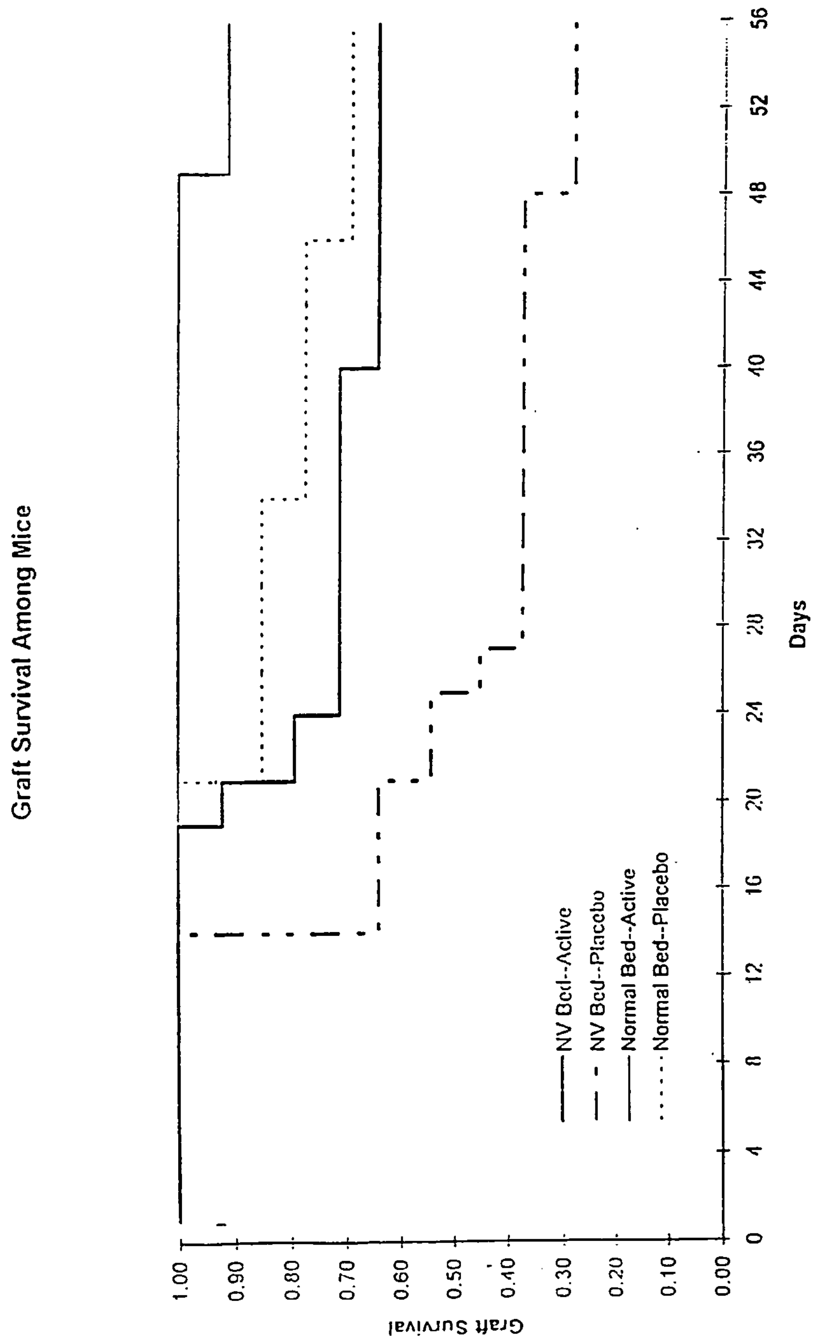

Graft Survival Among Mice
NV Bed--Active
NV Bed--Placebo
Normal Bed--Active
Normal Bed--Placebo
Graft Survival
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
Days

Fig. 2

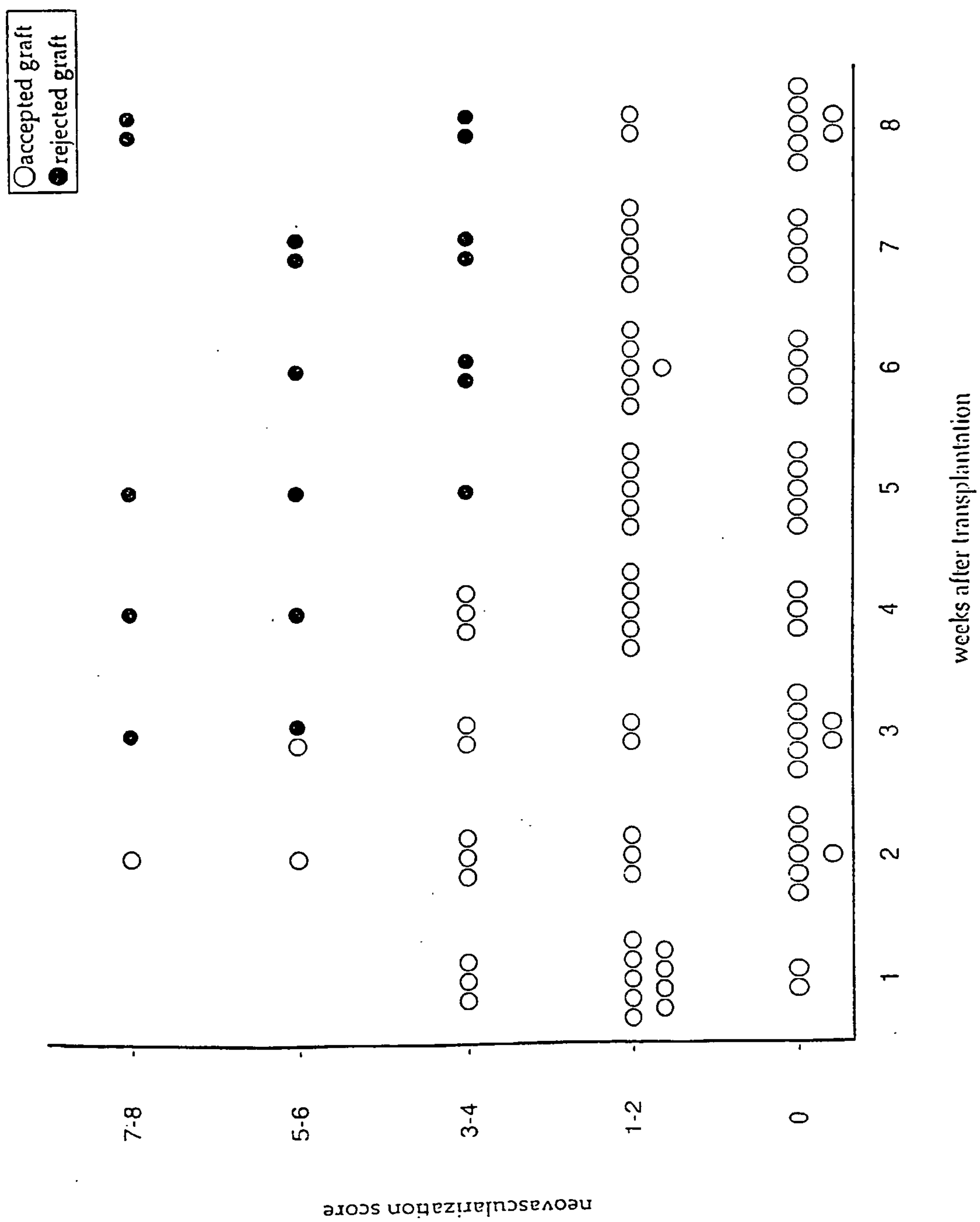

accepted graft
rejected graft
neovascularization score
7-8
5-6
3-4
1-2
0
1
2
3
4
5
6
7
8
weeks after transplantation

Fig. 3A

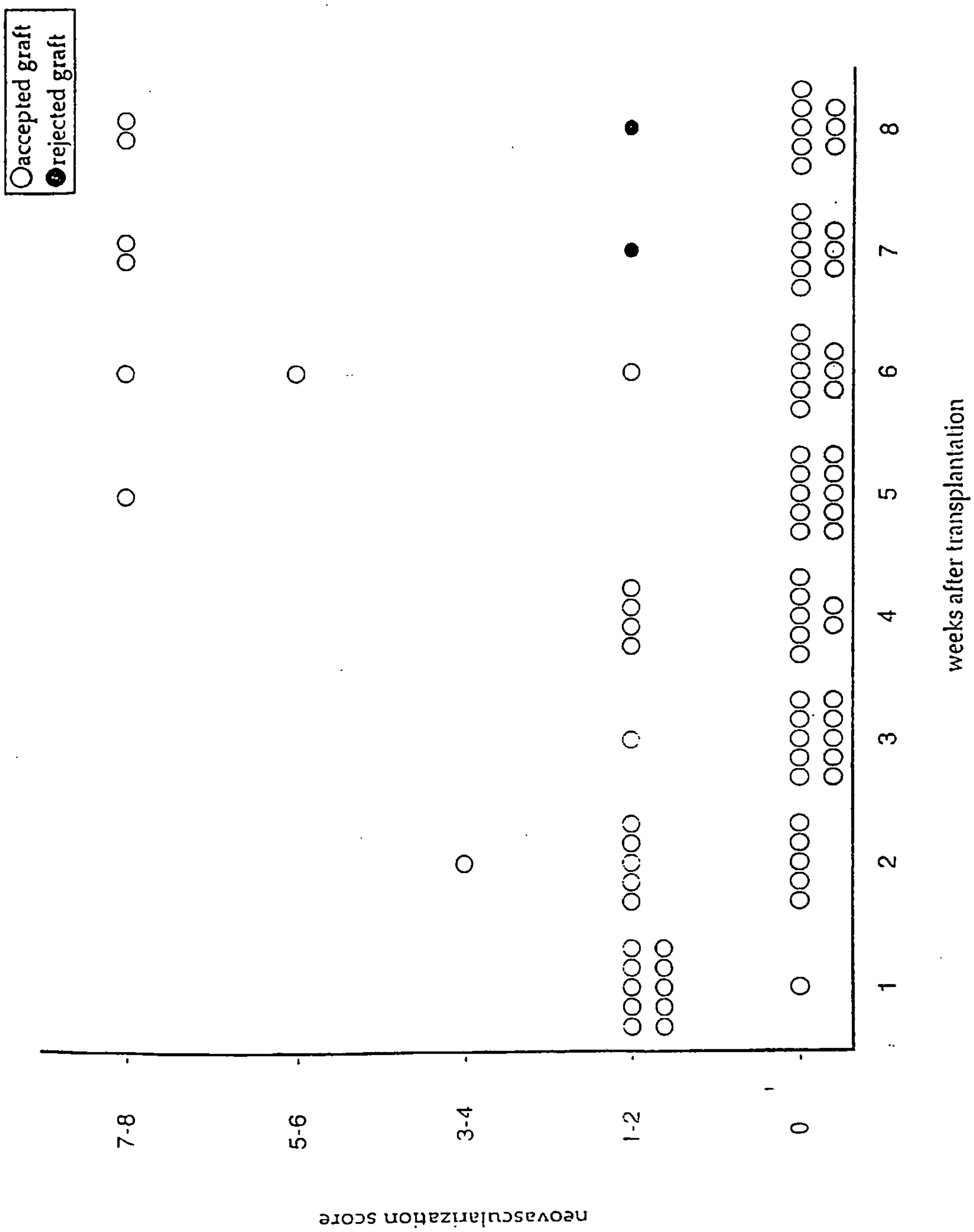
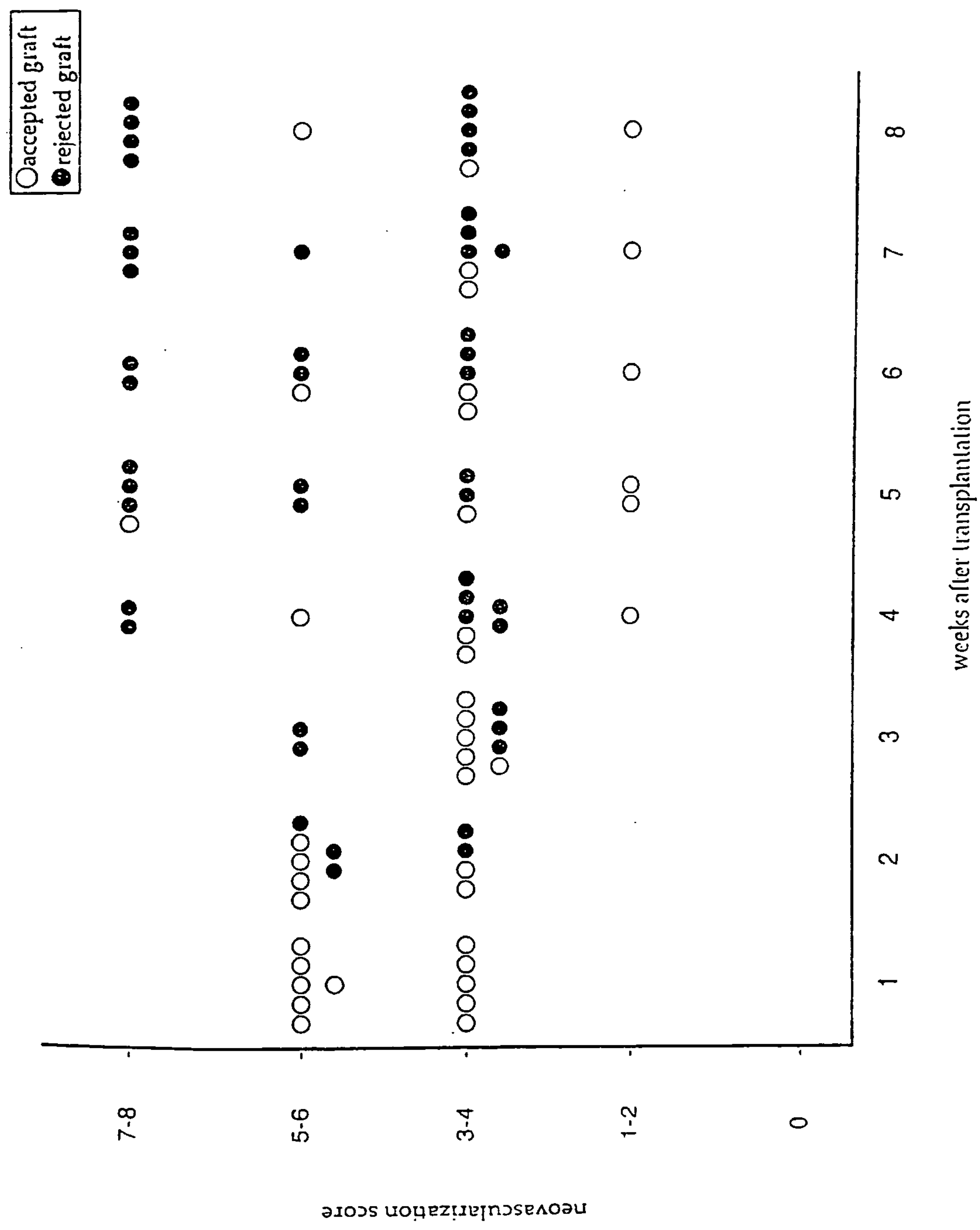

accepted graft
rejected graft
neovascularization score
7-8
5-6
3-4
1-2
0
weeks after transplantation
1
2
3
4
5
6
7
8

Fig. 3B


accepted graft
rejected graft
neovascularization score
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5-6
3-4
1-2
0
1
2
3
4
5
6
7
8
weeks after transplantation

Fig. 4A

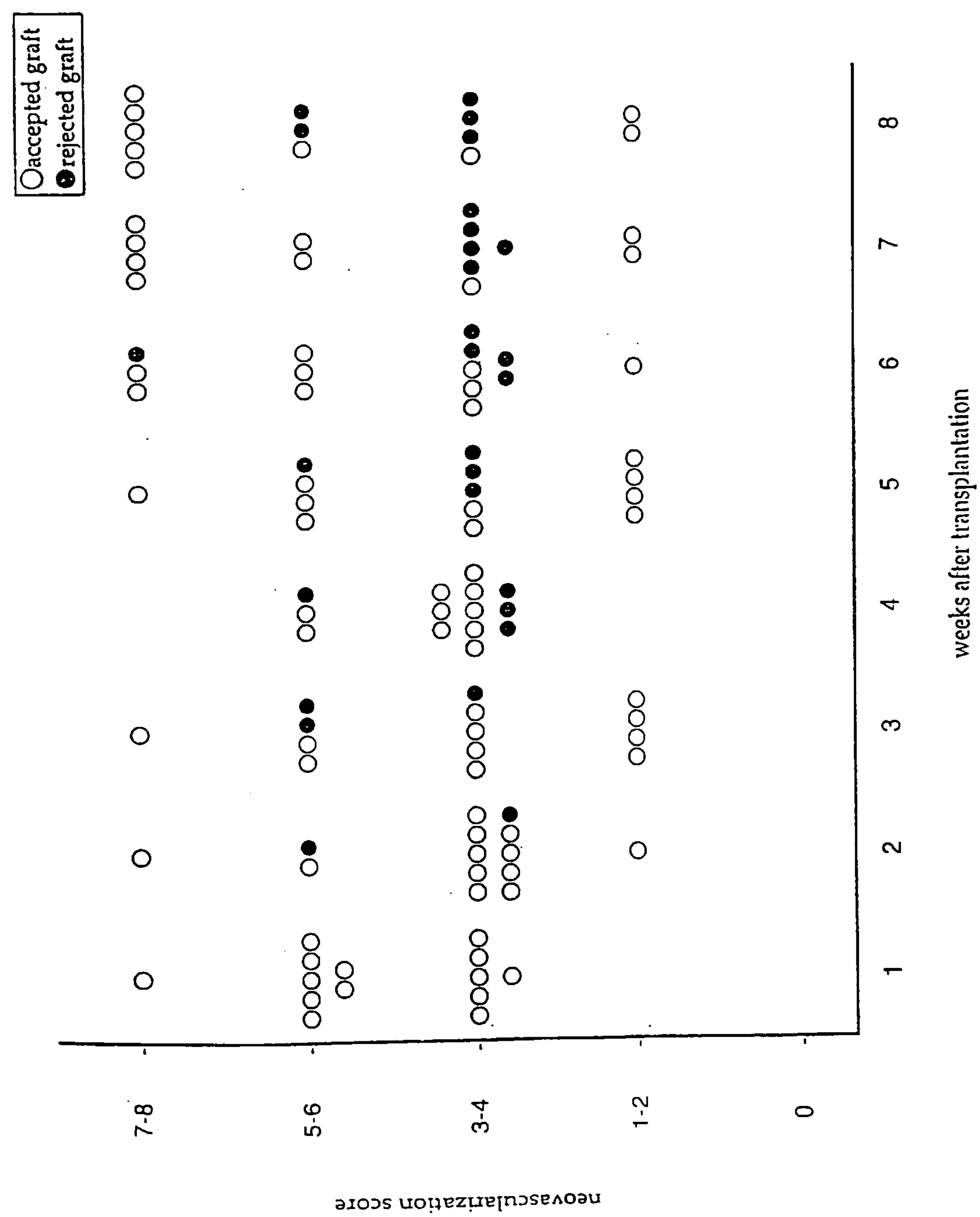
accepted graft
rejected graft
neovascularization score
7-8
5-6
3-4
1-2
0
1
2
3
4
5
6
7
8
weeks after transplantation

Fig. 4B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/21393

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 09323 A (DOMPE) 28 March 1996 see the whole document ---	1-20
X	ROSENBAUM, JAMES T. ET AL: "Activity of an interleukin 1 receptor antagonist in rabbit models of uveitis" ARCH. OPHTHALMOL. (CHICAGO) (1992), 110(4), 547-9, XP002059494 see the whole document ---	1-20

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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1

Date of the actual completion of the international search	Date of mailing of the international search report
19 March 1998	08.04.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreau, J

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US TORRES P F ET AL: "Cytokine mRNA expression during experimental corneal allograft rejection." XP002059499 see abstract & EXPERIMENTAL EYE RESEARCH 63 (4). 1996. 453-461,	1-20
A	KENNEDY M C ET AL: "Novel production of interleukin - 1 receptor antagonist peptides in normal human cornea." JOURNAL OF CLINICAL INVESTIGATION 95 (1). 1995. 82-88, XP002059496 see the whole document	1-20
A	TORRES P ET AL: "Interleukin 1-beta and interleukin 1 receptor antagonist expression during experimental corneal graft rejection." ANNUAL MEETING OF THE INVESTIGATIVE OPHTHALMOLOGY AND VISUAL SCIENCE, FORT LAUDERDALE, FLORIDA, USA, MAY 14-19, 1995. INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE 36 (4). 1995. S1009, XP002059497 see the whole document	1-20
P,X	DANA M R ET AL: "Topical interleukin - 1 receptor antagonist (IL - 1ra) suppresses Langerhans cell activity and promotes immune privilege." ANNUAL MEETING OF THE ASSOCIATION FOR RESEARCH IN VISION AND OPHTHALMOLOGY, PARTS 1-2, FORT LAUDERDALE, FLORIDA, USA, MAY 11-16, 1997. INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE 38 (4 PART 1-2). 1997. S705, XP002059495 see the whole document	1-20
P,X	DANA M R ET AL: "Topical interleukin 1 receptor antagonist promotes corneal transplant survival." TRANSPLANTATION (BALTIMORE) 63 (10). 1997. 1501-1507, XP002059498 see the whole document	1-20

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/21393

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-12,20
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-12,20
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/21393

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9609323 A	28-03-96	IT 1269989 B	16-04-97
		EP 0782584 A	09-07-97

CORRECTED VERSION*

CORRECTED VERSION**

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(81) Designated States: AU, BR, CA, CN, CZ, IL, JP, KR, MX, NO, SG, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: LOCAL USE OF IL–1RA IN CORNEAL TRANSPLANT REJECTION OR DISORDERS OF THE EYE

(57) Abstract

Topical application of interleukin–1 receptor antagonist (IL–1ra) is shown to promote corneal transplant survival in a murine model of orthotopic allotransplantation, having a significant effect in prolonging graft survival in both high–risk and normal (low–risk) stromal beds. Furthermore, the promotion of graft survival is associated with a significant decrease in corneal inflammation. Therefore, IL–1ra and related antagonists to interleukin–1 can be used in a therapeutic composition for topical prophylaxis and treatment of allograft rejection and for local treatment of a wide array of immunogenic inflammatory diseases of the eye. The composition comprises a therapeutically effective amount of IL–1ra in association with a pharmaceutically acceptable carrier vehicle for topical application.

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(57) Abstract

Topical application of interleukin-1 receptor antagonist (IL-1ra) is shown to promote corneal transplant survival in a murine model of orthotopic allotransplantation, having a significant effect in prolonging graft survival in both high-risk and normal (low-risk) stromal beds. Furthermore, the promotion of graft survival is associated with a significant decrease in corneal inflammation. Therefore, IL-1ra and related antagonists to interleukin-1 can be used in a therapeutic composition for topical prophylaxis and treatment of allograft rejection and for local treatment of a wide array of immunogenic inflammatory diseases of the eye. The composition comprises a therapeutically effective amount of IL-1ra in association with a pharmaceutically acceptable carrier vehicle for topical application.

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LOCAL USE OF IL-1RA IN CORNEAL TRANSPLANT REJECTION OR DISORDERS OF THE EYE

FIELD OF THE INVENTION

This invention relates to the prophylaxis and treatment of corneal transplant rejection and other immune and inflammatory disorders of the eye and more particularly to a topical treatment therefor.

GOVERNMENT RIGHTS

Part of the work leading to this invention was carried out with United States Government support provided under grants from the National Institutes of Health, Grant Nos. EY06622, EY00363 and EY19765. Therefore, the U.S. Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

Corneal transplantation has emerged as the most common and successful form of solid tissue transplantation with over 40,000 cases performed in the United States alone (1). In uncomplicated first allografts performed in avascular beds, the 2-year survival rate is over 90% (2). The extraordinary success of penetrating keratoplasty can be attributed to various features of the normal cornea and anterior segment that in the aggregate account for their "immune-privileged" state (3) including: (a) the avascularity of the stroma, (b) the absence of corneal lymphatics, (c) the rarity of indigenous professional antigen-presenting Langerhans cells (LC) or macrophages in the normal graft bed, (d) a unique spectrum of locally produced immunomodulatory cytokines that suppress immunogenic inflammation and complement activation (to which the cornea itself contributes), and (e) expression

of Fas ligand by these ocular tissues that can directly suppress immunogenic inflammation (4).

In spite of the overall success with corneal transplantation, however, a substantial percentage of corneal grafts experience at least one rejection episode. This is significant since of all the technical and tissue parameters that can affect final graft outcome, immunologic rejection represents the principal threat to allograft longevity regardless of the degree of allodisparity (5,6,7,8,9). This immunologic threat to graft survival is nowhere more evident than in vascularized recipient beds that tend to suffer from earlier and more fulminant rejection episodes that are more resistant to therapy (1,5,7,8,10).

The advent of corticosteroids and their use in the prophylaxis and treatment of corneal transplant rejections has represented the most significant contribution to the prolongation of corneal transplant survival over the last several decades (11,12). However, the local use of corticosteroids, or alternative general immunosuppressants, is associated with significant complications such as infection, cataracts, glaucoma and corneal thinning (13,14,15,16). General immunosuppressive therapy, when used systemically, may be associated with serious side-effects and multiorgan dysfunction (morbidity) which does at times culminate in death. It is therefore apparent that development of molecular strategies that can specifically target a critical step in the transplant rejection process is desirable and would prove to be an effective modality of circumventing the problems inherent in non-specific immune suppression.

SUMMARY OF THE INVENTION

Interleukin-1 (IL-1) is a potent proinflammatory cytokine that has a wide range of activities including the critical mediation of the acute-phase response, chemotaxis and activation of inflammatory and antigen-presenting cells,

upregulation of adhesion molecules/ costimulatory factors on cells, and stimulation of neovascularization (17,18,19,20). IL-1 has been implicated as an important cytokine in host immunologic reactions to a variety of non-ocular allografts (21,22,23). In the eye, IL-1 activity has been correlated with corneal neovascularization (24), endotoxin-mediated uveitis (25), corneal collagenase and metalloprotease expression (26,27), corneal injury in vitamin-A deficiency (28), and herpetic stromal keratitis (29). Niederkorn and co-workers have shown that IL-1 mediated Langerhans cell migration can play a critical role in host allosensitization in the setting of corneal transplantation (17,30). For all these reasons, IL-1 is an attractive target for therapeutic intervention in immunogenic inflammatory diseases.

Interleukin-1 receptor antagonist (IL-1ra) is a naturally occurring IL-1 isoform with high-affinity binding to both IL-1 receptor subtypes. IL-1ra functions as an active IL-1 inhibitor, having no agonist activity (31,32). There is a 77% homology between the predominant human and murine isoforms of IL-1ra, and systemic administration of recombinant human IL-1ra has been shown to have a profound downregulatory effect on the acute phase cytokine cascade in both man and mouse (33,34).

Others have attempted to assess the potential activity of IL-1ra in inhibiting the immunogenic effects of IL-1, with mixed results. Rosenbaum (57) reports that despite the activity of IL-1ra in inhibiting inflammation induced by the administration of IL-1 intravitreally in a New Zealand white rabbit model for uveitis, IL-1ra did not produce significant reduction in inflammation subsequent to an active Arthus reaction or subsequent to the intravitreal injection of E.coli endotoxin.

Nevertheless, it has surprisingly been found, and is reported here, that direct application of IL-1ra to corneal allografts leads to a significant prolongation of transplant survival. The results described below demonstrate that

IL-1ra administration has a significant positive effect in promoting corneal allograft survival, i.e., in increasing survival rates, of both normal- and high-risk transplant recipients. In addition, both normal- and high-risk IL-1ra treated graft sites had significantly less inflammation and Langerhans cell infiltration compared to untreated controls.

Therefore, the invention is directed to a method for treating allografts and preventing allograft rejection, or for generally treating an immune or inflammatory response of the eye. The method of the invention includes direct, local application of a therapeutic composition to an affected area of a patient. The therapeutic composition useful in the method of the invention comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for local application. Furthermore, the therapeutic composition can be packaged as an article of manufacture of the invention that includes a label indicating the use of the composition in the method of the invention. Preferably, the interleukin-1 antagonist is an interleukin-1 receptor antagonist and, most preferably, the naturally occurring (or recombinant) human IL-1 isoform IL-1ra. Alternatively, other interleukin-1 antagonists may be utilized for the same effect. These include, but are not limited to, (1) modifications of native IL-1ra that would, e.g., render this compound more bioactive, or (2) other IL-1 antagonists that would bind and hence render inactive the IL-1 receptors (e.g., anti-IL-1 receptor antibodies) and/or (3) soluble form(s) of the IL-1 receptor that would bind IL-1 isoforms and prevent their binding to cell-associated receptors. The carrier vehicle in the composition of the invention is preferably a viscous formulation, and most preferably, sodium hyaluronate for application to the corneal surface, to promote a longer residence time for the therapeutic agent at the affected site of the patient. Furthermore, in another method of the invention, sodium hyaluronate (or any other appropriate

hyaluronate salt) can be used as a pharmaceutical carrier vehicle for the delivery of other therapeutic agents to the ocular surface of a patient.

Preferably, the method of the invention is used to prolong transplant survival in corneal allograft recipients. The method of the invention would also be useful for therapeutic intervention in immunogenic inflammatory diseases of the cornea and ocular surface, such as keratoconjunctivitis sicca and other dry eye states including Sjögren's syndrome, allergic conjunctivitis and other atopic conditions of the ocular surface, corneal neovascularization, and immune or infectious keratitis states. In addition, upon local injection or irrigation, the method of the invention would be useful for suppressing diseases such as uveitis and post-surgical inflammation in intraocular compartments (e.g., anterior chamber or vitreous cavity). Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a conceptual relationship among corneal neovascularization, IL-1ra treatment, and graft outcome based on degree of preoperative risk;

Fig. 2 shows Kaplan-Meier survival curves for normal-risk and high-risk corneal allograft recipients;

Figs. 3A and 3B show association between corneal allograft survival and neovascularization score in normal-risk recipients based on IL-1ra treatment; and

Fig. 4A and 4B show association between corneal allograft survival and neovascularization score in high-risk recipients based on IL-1ra treatment.

DETAILED DESCRIPTION OF THE INVENTION

The currently available pharmaceutic armamentarium for corneal transplant survival is primarily composed of

corticosteroids. Their introduction into ophthalmology is arguably the single most significant factor in the last four decades' advances in corneal transplant surgery (13). Nevertheless, beyond their well-known serious complications, corticosteroids show widely variable efficacy in preventing ultimate immunogenic graft failure, and this is particularly the case in high-risk keratoplasty (1,7). This series of experiments was conducted to test whether the specific inhibition of the important proinflammatory cytokine IL-1, by application of IL-1 receptor antagonist (IL-1ra), could be successful in prolonging either normal- or high-risk orthotopic corneal allografts in the mouse.

For all experiments, C57BL/6 corneas were transplanted into BALB/c (major histocompatibility [MHC] and minor H-disparate) eyes. "High-risk" transplants consisted of transplants that were sutured into BALB/c recipient beds with corneal neovascularization induced by placement of three interrupted sutures in the host cornea two weeks previously. Both risk groups were divided in a masked fashion into treatment subgroups that received either 20mg/ml of IL-1ra mixed in 0.2% sodium hyaluronate vehicle (N=28) or placebo alone (N=25). All transplants were evaluated for 8 weeks postoperatively for signs of rejection. Any changes in the degree of corneal neovascularization were also determined. At the end of follow-up, corneal specimens were processed for enumeration of Langerhans cells and for histopathological evaluation.

The results show a significant increase in the survival rates of both normal- and high-risk transplants among the IL-1ra-treated animals compared to untreated controls by both stratified Mantel-Haenszel (P=0.02) and Kaplan-Meier survival (P=0.03) analyses. Furthermore, both normal- and high-risk IL-1ra treated grafts have significantly less inflammation and Langerhans cells infiltration compared to untreated controls.

There is little doubt that presence of corneal neovascularization is a significant risk factor for corneal allograft survival (1,6,7,10,36). Therefore, the relationship between IL-1ra treatment and neovascularization scores was also examined. In the normal-risk, but not in the high-risk setting where neovascularization had been induced two weeks previously, IL-1ra treatment was determined to be associated with a blunted postkeratoplasty neovascularization response. Laboratory results show that IL-1ra can significantly blunt the <u>early</u>, but not late, phase corneal neovascularization development in response to standard angiogenic stimuli, suggesting that there are non-IL-1 mediated factors that can overshadow IL-1 suppression in corneal angiogenesis. The failure of IL-1ra to lead to significant neovascularization <u>regression</u> in the high-risk beds, as opposed to its capacity for angio<u>stasis</u> in the normal-risk beds as demonstrated here, is apparently due to the dominance of non-IL-1 driven angiogenic factors in the former. However, IL-1ra appears to play an important role, possibly in combination with other agent(s) in suppressing the neovascular response.

In the aggregate, development of corneal neovascularization causes sufficient perturbation of the ocular microenvironment to lead to a loss of "immune privilege" as measured by the ability to induce anterior chamber-associated immune deviation (ACAID) (35). However, in contrast to the expectation that the efficacy with which IL-1ra could blunt rejection in the high-risk corneas would be paralleled by an equal degree of suppression in corneal neovascularization in the high-risk setting, it was determined that treatment with IL-1ra promotes allograft survival in recipients regardless of their neovascularization status. This effect could mirror what has been described previously in neovascularized corneas where therapeutic measures that have been shown to restore immune

privilege/ACAID are associated with highly variable degrees of angiostasis (35).

From the results described above, it appears that an interrelationship exists among corneal neovascularization, IL-1ra treatment and graft outcome based on the degree of preoperative risk. Referring to Fig. 1, in normal-risk (or virgin) corneal beds **10**, suppression of IL-1 activity by IL-1ra treatment **12** has a significant dampening effect on corneal angiogenesis, reducing neovascularization to a mild (0/++NV) or minimal (0/+NV) degree **14**. Furthermore, treatment with IL-1ra **12** has a significant effect on promoting graft longevity **16**. In the neovascularized cornea **18**, the effect of IL-1ra treatment **20** on corneal transplantation **16** appears to be independent of the degree of corneal neovascularization, which remains at the significant (+++NV) level **22**. Specifically, IL-1ra treatment of high-risk beds (with antecedent neovascularization) does not appear to significantly suppress neovascularization; however, this same treatment leads to significant prolongation of graft survival compared to untreated controls.

The degree to which the migration of Langerhans cells (LC) into the central cornea can be blunted by application of IL-1ra was intriguing. Since the healthy and unoperated cornea is essentially devoid of these constitutively antigen-presenting cells as well as other MHC class II-bearing "passenger leukocytes," the presence of LC in the central cornea has been implicated in the loss of local immune privilege, by virtue of their critical role in immune surveillance and allosensitization in the "indirect pathway" (2,17,36). IL-1 has been shown to be a critical regulator of LC migration in the cornea (17), and the activity of epidermal LC is known to be at least partially controlled by IL-1 (30,49). Hence, the demonstrated constitutive expression by normal corneal cells of IL-1ra (50) likely plays an important immune regulatory role in the

avascular/non-traumatized cornea by keeping the microenvironment in an inhospitable site for sensitization. The results reported here, showing a decrease in LC numbers in IL-1ra treated corneas compared to untreated controls, are evidence that in traumatized corneas the induction of allosensitization in the corneal allograft can be tilted in favor of unresponsiveness by the application of high-doses of IL-1ra. This effect is reflected in greater longevity of these allografts.

The specific regimen utilized in these studies for the delivery of IL-1ra to the cornea was based on the observation that ocular bioavailability of topical medications is enhanced in viscous formulations (51,52). Traditional formulations that rely on aqueous drops for topical treatment often provide low bioavailability because of efficient elimination processes active on the ocular surface which typically lead to a very short drug residence time. The choice of sodium hyaluronate (SH) as the preferred vehicle in this series of experiments was based on previous observations that 0.2% SH has a very long contact (residence) time on the ocular surface. This vehicle is also well-tolerated due to its pseudoplastic biophysical properties that offer little resistance to high shear rates (52,53).

The following examples are presented to illustrate the advantages of the present invention and to assist one of ordinary skill in making and using the same. These examples are not intended in any way otherwise to limit the scope of the disclosure.

EXAMPLE I

Corneal Transplant Survival Following IL-1ra Treatment

Fifty-five corneal allografts were performed in 55 BALB/c mice, of which 53 were deemed technically acceptable for long-term follow-up; that is, anterior segment integrity was maintained with no signs of wound leak, infection, or

hyphema. These were subdivided, based on degree of immunologic risk as described above, into normal- (N=28) and high-risk (N=25) groups.

Topical preparations of the therapeutic agent were applied to the recipient mice three times daily for the 56 days (8 weeks) duration of the study. The study medication was composed of 20 mg/ml of human recombinant IL-1ra in 0.2% sodium hyaluronate in PBS (supplied by Amgen, Boulder, CO). Placebo-treated animals received the vehicle 0.2% sodium hyaluronate only. Graft success or failure was established based on opacity scores, as detailed in Materials and Methods below.

Statistical analysis of cumulative rejection rates, after stratification for degree of risk based on recipient bed vascularity, revealed a strong association between IL-1ra treatment and graft survival (Mantel-Haenszel test, $P=0.02$). The 8-week incidences of transplant rejection were lowest in the normal-risk grafts that were treated with IL-1ra (7%), and highest in the high-risk grafts that received vehicle only (73%) (Table I).

Table I

Corneal transplant rejection rates, stratified by IL-1a therapy and degree of risk.

Degree of Risk	N	Rejection rate*	Rejection Reaction rate$^{\Psi}$
Normal Risk			
treated	14	7%	7%
untreated	14	29%	50%
High Risk↑			
treated	14	36%	43%
untreated	11	73%	91%
Total			
treated	28	21%	25%
untreated	25	48%	68%

* Cumulative rejection rate over 8-week follow-up period.

Ψ Includes opacification score of ≥ 2+ at any time point after 2 weeks, as described in Materials and Methods.

↑ High-risk transplants are by definition grafted into vascularized stromal beds, as described in Materials and Methods.

Four of the animals used developed dystrophic/degenerative corneal calcific deposits following surgery. Because of the reported association between this common BALB/c corneal finding and loss of immune privilege in the anterior segment (39), these animals were censored from further evaluation prior to completion of the 8-week follow-up course. To prevent generation of bias by artificially lowering the denominator size in 8-week rate calculations, survival curves were developed. Referring to Fig. 2, Kaplan-Meier survival curves for normal-risk (N=28) and high-risk (N=25) corneal allograft recipients are shown, stratified by treatment with IL-1ra active agent or placebo. Survival analysis revealed that IL-1ra treatment was associated with significant graft longevity in both normal-risk (P=0.1) and high-risk (P<0.05) recipient beds, with an overall reduction in rejection rate of 56% (P=0.03). Thus, in both cases there is an association between IL-1ra treatment and transplant outcome, as survival rates of high-risk grafts treated with the active agent closely mirror those of normal-risk transplants receiving placebo.

EXAMPLE II

Corneal Neovascularization

In addition to graft survival/opacification criteria detailed in Materials and Methods, transplants were also followed biomicroscopically for the degree of corneal neovascularization. All high-risk beds had been specially prepared for two weeks to develop two or more quadrants of stromal neovascularization as described previously (36), and all normal-risk corneal beds were avascular.

It has been shown previously, in both man (40) and mouse (37), that corneal transplantation alone can induce neovascularization. Since post-keratoplasty corneal neovascularization likely plays an important role in facilitating effector elements in the inflamed cornea (35,40), the corneas were also examined to see if treatment

with IL-1ra had an appreciable effect on this parameter, and an angiostatic effect with IL-1ra treatment in the normal-risk, but not high-risk, transplants was observed.

Referring to Figs. 3A and 3B, among the normal-risk grafts, 38% of the untreated corneas (Fig. 3A) had a neovascularization score of ≥3 at 4 weeks compared to none of the IL-1ra treated cases (Fig. 3B). Respective rates at 8 weeks were 31% for untreated controls and 18% for treated cases. In contrast, referring to Figs. 4A and 4B, no significant association of angiostatic effect with IL-1ra treatment was apparent in high-risk eyes that had been induced to have corneal neovascularization two weeks previously. The proportions of corneas with a neovascularization score of ≥3 at 4 and 8 weeks follow-up was very comparable between untreated controls (Fig. 4A) (91% at both time points) and treated cases (Fig. 4B) (100% and 86% at respective time points).

Furthermore, there was a significant correlation between corneal angiogenesis and rejection in both untreated normal- and high-risk controls. Among untreated normal-risk transplants (Fig. 3A), 4 of 4 corneas with a neovascularization score of ≥3 had rejected at 8 weeks. Similarly, among untreated high-risk recipients (Fig. 4A), 7 of 10 grafts with a neovascularization score of ≥3 had rejected at 4 weeks, and 8 of 10 grafts with neovascularization ≥3 had rejected at 8 weeks follow-up. In contrast, there was a distinct divergence between corneal angiogenesis and graft survival among both normal- and high-risk transplants treated with IL-1ra. For example, among the normal-risk transplants that had received treatment with IL-1ra (Fig. 3B), the one allograft that rejected at 8 weeks had minimal neovascularization, and two treated grafts with significant neovascularization never rejected. Similarly, among the treated high-risk transplants (Fig. 4B), 5/12 grafts with significant neovascularization had rejected at

8 weeks, and almost the same proportion (7/12) had not rejected.

EXAMPLE III

Langerhans Cell Population.

The presence of Langerhans cells (LC) in the cornea has been associated with immunogenic inflammation, the host's ability to be allosensitized, and loss of immune privilege (39,41,42). To explore this point further, naive age-matched BALB/c corneas and allografts were excised at the completion of the follow-up period to assay their LC populations with fluorescence microscopy, as described in Materials and Methods.

Consistent with previous findings, the central and paracentral areas of normal naive and avascular corneas had very few LC. Allogeneic transplants led to a significant increase in the number of LC in the central portions of the cornea. Interestingly, however, treatment with IL-1ra had a significant dampening effect on LC migration, regardless of the degree of pre- or postoperative corneal neovascularization. Among the normal-risk allografts, the average number of central corneal LC in the IL-1ra treated corneas was $13/mm^2$ compared to $41/mm^2$ (32%) in the untreated controls (P=0.03). A similar reduction in the number of LC was observed after IL-1ra treatment in the vascularized high-risk recipient beds where the number of LC was $27/mm^2$ in the treated corneas compared to $89/mm^2$ in the untreated eyes (P=0.02).

EXAMPLE IV

Corneal Inflammation

Histopathological evaluation of IL-1ra treated and untreated corneas, at 8 weeks, in both normal- and high-risk eyes demonstrated a noticeable decrease in the number of infiltrating leukocytes (particularly neutrophils) into grafts that had undergone treatment, with an associated decrease in the degree of stromal edema. In spite of

comparable degrees of clinically evident corneal neovascularization in the untreated and treated high-risk grafts, as described above, there was still an appreciable difference in the level of neutrophil infiltration between treated corneas and vehicle-treated controls. The decreased corneal inflammation in the IL-1ra-treated allografts was reflected by a generally lower opacity score (irrespective of final rejection status) in the IL-1ra-treated transplants. For example, all but one of the untreated normal-risk grafts that eventually failed developed opacity scores ≥3; whereas the single IL-1ra treated normal-risk graft that failed had an opacity score of 2.

Materials and Methods

Mice and anesthesia. Six to ten-week old BALB/c (H-2^d) and C57BL/6 (H-2^b) mice were purchased (Taconic, New York) or obtained from the Schepens Eye Research Institute animal colony. All animals were treated according to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Each animal was deeply anesthetized with an intramuscular injection of 3 to 4 mg ketamine and 0.1 mg xylazine prior to all surgical procedures.

Corneal transplantation. All 55 transplants involved combined MHC- and minor alloantigen disparate corneas that were grafted from C57BL/6 donors into BALB/c eyes as follows. On day 0, in each case, the central 2-mm of the donor cornea was marked with a microcurrette and the donor button excised by Vannas scissors and placed in phosphate-buffered saline (PBS). The recipient graft bed was prepared by excising the central 2-mm of the cornea. The donor button was then secured in place with 8 interrupted 11-0 nylon sutures (Sharppoint; Vanguard, Houston, TX). Antibiotic ointment was applied to the corneal surface and the lids were shut for 24 hours with an 8-0 nylon tarsorrhaphy for the next day after

which treatment would start. Animals were divided in a masked fashion into cases that would receive active IL-1ra and controls that would receive vehicle/placebo alone as detailed below. Grafted eyes with technical difficulties (hyphema, infection, or loss of anterior chamber) were excluded from study. Transplant sutures were removed in all cases on day 7.

Induction and grading of corneal neovascularization. Intrastromal sutures induce robust neovascularization growth into the normally avascular corneal stroma from the limbus that can be appreciated as early as three days following suture placement (35), and untreated allografts into these high-risk beds are rejected swiftly (36). Two parallel protocols were devised to study normal- and high-risk corneal transplantation. In the former case, animals were left unmanipulated until the day of surgery. High-risk beds were developed as described previously (36). Briefly, three interrupted 11-0 sutures were placed in the central cornea of one eye of a normal BALB/c mouse on day -14 under aseptic microsurgical technique using an operating microscope. The neovascularized beds then served as high-risk graft beds for orthotopic corneal transplants on day 0 as described above (neovascularization-inducing sutures were removed at the time of transplantation). Neovascularization was graded between 0-8 as described previously based on the degree of centripetal ingrowth and quandrantic involvement of the neovessels (37).

Evaluation and scoring of orthotopic corneal transplants. Grafts were evaluated by slitlamp biomicroscopy twice a week. At each time point grafts were scored for opacification. A previously described scoring system (37) was used to measure the degree of opacification between 0-5+: 0=clear and compact graft; 1+=minimal superficial opacity; 2+=mild deep (stromal) opacity with pupil margin and iris

vessels visible; 3+=moderate stromal opacity with only pupil margin visible; 4+=intense stromal opacity with the anterior chamber visible; 5+=maximal corneal opacity with total obscuration of the anterior chamber. Grafts with an opacity score of 2+ or greater after three weeks were considered as rejected (immunologic failure); grafts with an opacity score of 3+ or greater at two weeks that never cleared were also regarded as rejected. Since some grafts had only transient opacification, grafts with an opacity score of 2+ or greater at any time point after two weeks were considered to have a rejection reaction (RR), regardless of the opacity score at eight weeks (37).

Langerhans cell (LC) enumeration and histopathological evaluation. The LC were assessed by an immunofluorescence assay performed on whole corneal epithelial mounts as previously described (38). Briefly, each eye was enucleated and the anterior segment dissected under the operating microscope. The cornea was placed in 20mM ethylenediaminetetraacetic acid (EDTA) buffer and incubated for 30 minutes at 37°C, followed by removal of the epithelium *in toto* and washed in PBS at room temperature. The cornea was then fixed with 95% ethanol prior to washing and incubation with 1:20 diluted primary anti-murine Ia^d antibody for 45 minutes at 37°C. The tissue was then washed in PBS and incubated with a FITC-labeled goat anti-mouse secondary antibody for 30 minutes at 37°C. Negative controls either bypassed this step or were incubated with antibody specific for an unrelated MHC epitope. Sections were then mounted on slides and examined under the fluorescent microscope with a square ocular grid where LC were enumerated. Corneal specimens that were not processed for LC enumeration were fixed, sectioned, and stained with hematoxylin-eosin for light microscopic evaluation.

Statistical techniques. The proportional rates of rejected allografts in the IL-1ra and vehicle-only treated groups were compared using two methods. First, the Mantel-Haenszel summary chi-square statistic was obtained, stratified by (adjusted for) degree of preoperative risk (i.e., normal vs. vascularized stromal bed) to compare the proportion of rejected transplants in the two groups. Second, Kaplan-Meier survival curves were constructed in order to compare the probability of graft survival over the follow-up period, both overall and separately for normal- and high-risk eyes, in the IL-1ra treated and untreated controls. This method accounted for the variability in the time-to-graft rejection in addition to the variation in follow-up time (4 mice in the normal-risk group had follow-up terminated prior to the end of the 8-week period). Comparison of Langerhans cell population means among IL-1ra treated eyes and untreated controls was made by the Student's t-test.

Use

IL-1ra is a very promising agent for use in corneal transplantation, both because of its efficacy as demonstrated in these experimental results and its putative value over existing therapy, which has well-known side-effects and complications. In addition, the very significant dampening of the inflammatory response observed suggests that treatment with IL-1ra and other antagonists of IL-1 and its receptors can be applied to a wide variety of ocular immune and inflammatory disorders.

IL-1 antagonism, e.g., via use of IL-1ra, can suppress immunogenic inflammation, as demonstrated in the corneal transplant model herein, in both virgin and previously inflamed/neovascularized eyes. In the eye, the topical administration of IL-1ra can include non-transplant therapeutic uses such as treatment of allergic and hypersensitivity disorders of the ocular surface, burns,

infections, dry eye disorders, and chronic inflammatory states that may lead to neovascularization and/or scarring or fibrosis of the cornea and ocular surface. In addition to sodium hyaluronate, other vehicles, e.g., cyclodextrins may be used to increase drug deliver to the surface epithelium.

The ocular use of IL-1ra is not limited to topical administration to the cornea and ocular surface. Intraocular administration, e.g., by intraocular injection into the anterior chamber or irrigation at the time of surgery, is appropriate for treatment (or prophylaxis of recurrence) of intraocular inflammatory disorders such as autoimmune or infectious uveitis, post-traumatic or post-surgical inflammation, or idiopathic uveitides. Sustained release formulations, e.g., with use of biodegradable or non-degradable biocompatible polymers, or simple irrigation of these agent(s) at the time of surgery, can be used for intraocular delivery of IL-1ra to subjects.

Other candidate interleukin-1 antagonists that might be useful in the methods of the invention, as described earlier, can be tested for effectiveness using one of the assays described herein (e.g., measuring the extent of corneal inflammation, neovascularization, graft survival or Langerhans cell migration) and the results compared to those obtained with IL-1ra.

The dosage of IL-1ra used in the experiments described herein was relatively high in order to determine the maximum positive effect of treatment. However, IL-1ra appears to be able to exert its suppressive effect over a wide dose range (56). Optimal dosage and appropriate modes of administration for each of the conditions delineated above can be determined by conventional protocols. For example, in the case of corneal transplantation, other doses ranging between 20ng/ml - 2mg/ml will additionally be tested and the endpoints described above (e.g., effect on corneal inflammation, neovascularization, graft longevity or Langerhans cell

migration) for the tested dosage will be compared to those obtained using the current dose herein of 20mg/ml. It is to be expected that an appropriate concentration of an IL-1 receptor antagonist in a vehicle for local administration to a human patient will be in the range of 20ng/ml to 20 mg/ml.

References:

1. The Collaborative Corneal Transplantation Studies Research Group, The collaborative corneal transplantation studies (CCTS); "Effectiveness of histocompatibility matching in high-risk corneal transplantation," Arch. Ophthalmol. 110:1392 (1992).

2. Niederkorn, "Immune privilege and immune regulation in the eye," Adv. Immunol. 48:191 (1990).

3. Streilein, "Immunological non-responsiveness and acquisition of tolerance in relation to immune privilege in the eye," Eye 9:236 (1995).

4. Griffith et al., "Fas ligand-induced apoptosis as a mechanism of immune privilege," Science 270:1189 (1995).

5. Mader et al., "The high-risk penetrating keratoplasty," Ophthalmol Clin. North Am. 4:411 (1991).

6. Coster, "Mechanisms of corneal graft failure: the erosion of corneal privilege," Eye 2:251 (1989).

7. Maguire et al, "Risk factors for corneal graft failure and rejection in the collaborative corneal transplantation studies," Ophthalmology 101:1536 (1994).

8. Williams et al., "Factors predictive of corneal graft survival; Report from the Australian Corneal Graft Registry, Ophthalmology 99:403 (1992).

9. Alldredge et al., "Clinical types of corneal transplant rejection. Their manifestations, frequency, preoperative correlates, and treatment," Arch. Ophthalmol. 99:599 (1981).

10. Volker et al., "Hierarchy of prognostic factors for corneal allograft survival," Austr. NZ J. Ophthalmol. 15:11 (1987).

11. Wilson et al., "Graft failure after penetrating keratoplasty," Surv. Ophthalmol. 34:325 (1990).

12. Hill et al., "Corticosteroids in corneal graft rejection. Oral versus single pulse therapy," Ophthalmology 98:329 (1991).

13. Raizman, "Corticosteroid therapy of eye disease. Fifty years later," Arch. Ophthalmol. 114:1000 (1996).

14. Hemady et al., "Immunosuppressive drugs in immune and inflammatory ocular disease," Surv. Ophthalmol. 35:369 (1991).

15. Barraquer, "Immunosuppressive agents in penetrating keratoplasty," Am. J. Ophthalmol. 100:61 (1985).

16. Frangie et al., "Steroids," Int. Ophthalmol. Clin. 33:9 (1993).

17. Niederdorn et al., "Phagocytosis of particulate antigens by corneal epithelial cells stimulates interleukin-1 secretion and migration of Langerhans cells into the central cornea," Reg. Immunol. 2:83 (1989).

18. Dinarello et al., "The role of interleukin-1 in disease," New Eng. J. Med. 328:106 (1993).

19. Le et al., "Tumor necrosis factor and interleukin 1: cytokines with multiple overlapping biological activities," Lab. Invest. 56:234 (1987).

20. De Vos et al., "Cytokines and uveitis, a review," Curr. Eye Res. 11:581 (1992).

21. Buchwald et al., "Clinical value of interleukin 1- and interleukin 2-determinations in patients after kidney transplantation," Allergie und Immunologie 36:137 (1990).

22. Takasu et al., "A new immunosuppressant, 15-deoxyspergualin, inhibits production of IL-1 from isolated hepatic sinusoidal lining cells in swine liver transplantation," Transplant Proc. 21:1081 (1989).

23. Tilg et al., "Evaluation of cytokines and cytokine-induced secondary messages in sera of patients after liver transplantation," Transplantation 49:1074 (1990).

24. BenEzra et al., "In vivo angiogenic activity of interleukins," Arch. Ophthalmol. 108:573 (1990).

25. Kijlstra, "The role of cytokines in ocular inflammation," Br. J. Ophthalmol. 78:885 (1994).

26. Girard et al., "Transforming growth factor-beta and interleukin-1 modulate metalloproteinase expression by corneal stromal cells," Invest. Ophthalmol. Vis. Sci. 32:2441 (1991).

27. West-Mays et al., "Competence for collagenase gene expression by tissue fibroblasts requires activation of an interleukin 1 alpha autocrine loop," Proc. Natl. Acad. Sci. USA 92:6768 (1995).

28. Shams et al., "Increased interleukin-1 activity in the injured vitamin A-deficient cornea," Cornea 13:156 (1994).

29. Staats et al. "Cytokine expression in vivo during murine herpetic stromal keratitis. Effect of protective antibody therapy," J. Immunol. 151:277 (1993).

30. Niederkorn, "Effect of cytokine-induced migration of Langerhans cells on corneal allograft survival," Eye 9:215 (1995).

31. Hannum et al., "Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor," Nature 343:336 (1990).

32. Eisenberg et al., "Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist," Nature 343:341 (1990).

33. Antin et al., "Recombinant human interleukin-1 receptor antagonist in the treatment of steroid-resistant graft-versus-host disease," Blood 84:1342 (1994).

34. Ohlsson et al., "Interleukin-1 receptor antagonist reduces mortality from endotoxin shock," Nature 348:550 (1990).

35. Dana et al., "Loss and restoration of immune privilege in eyes with corneal neovascularization," Invest. Ophthalmol. Vis. Sci. 37:in press (1996).

36. Sano et al., "Fate of orthotopic corneal allografts in eyes that cannot support anterior chamber-associated immune deviation induction," Invest. Ophthalmol. Vis. Sci. 36:2176 (1995).

37. Sonoda et al. "Orthotopic corneal transplantation in mice--evidence that the immunogenetic rules of rejection do not apply," Transplantation 54:694 (1992).

38. Gillette et al., "Langerhans cells of the ocular surface," Ophthalmology 89:700 (1982).

39. Williamson et al., "Immunobiology of Langerhans cells on the ocular surface. I. Langerhans cells within the central cornea interfere with induction of anterior chamber associated immune deviation," Invest. Ophthalmol. Vis. Sci. 28:1527 (1987).

40. Dana et al., "Corneal neovascularization after penetrating keratoplasty," Cornea 14:604 (1995).

41. McLeish et al., "Immunobiology of Langerhans cells on the ocular surface. II. Role of central corneal Langerhans cells in stromal keratitis following experimental HSV-1 infection in mice," Reg. Immunol. 2:236 (1989).

42. Van der Veen et al., "Prevention of corneal allograft rejection in rats treated with subconjunctival injections of liposomes containing dichloromethylene diphosphonate," Invest. Ophthalmol. Vis. Sci. 35:3505 (1994).

43. Streilein et al., "Immunosuppressive properties of tissues obtained from eyes with experimentally manipulated corneas," Invest. Ophthalmol. Vis. Sci. 37:413 (1996).

44. Briscoe et al., "Antigen-dependent activation of T helper cell subsets by endothelium," Transplantation 59:1638 (1995).

45. Pober et al., "Immunologic interactions of T lymphocytes with vascular endothelium," Adv. Immunol. 50:261 (1991).

46. Collin, "Corneal lymphatics in alloxan vascularized rabbit eyes," Invest. Ophthalmol. 5:1 (1966).

47. Pabilack et al., "Differential expression of human corneal and perilimbal ICAM-1 by inflammatory cytokines," Invest. Ophthalmol. Vis. Sci. 33:564 (1992).

48. Gerritsen et al., "Cytokine activation of human macro- and microvessel-derived endothelial cells," Blood Cells 19:325 (1993).

49. Heufler et al., "Granulocyte/macrophage colony-stimulating factor and interleukin 1 mediate the maturation of murine epidermal Langerhans cells into potent immunostimulatory dendritic cells," J. Exp. Med. 167:700 (1988).

50. Kennedy et al., "Novel production of interleukin-1 receptor antagonist peptides in normal human cornea," J. Cl. Invest. 95:82 (1995).

51. Burstein, "Basic science of ocular pharmacology," In: Bartlett JD, Jaanus SD, eds. Clinical Ocular Pharmacology, 2nd ed. Boston: Butterworth 3 (1989).

52. Saettone et al., "The effect of different ophthalmic vehicles on the activity of tropicamide in man," J. Pharm. Pharmacol. 32:519 (1980).

53. Snibson et al., "Ocular surface residence times of artificial tear solution," Cornea 11:288 (1992).

54. Sand et al., "Sodium hyaluronate in the treatment of keratoconjunctivitis sicca. A double masked clinical trial," Acta. Ophthalmol. 67:181 (1989).

55. Shams et al., "Interferon-gamma, Staphylococcus aureaus, and lipopolysaccharide/silica enhance interleukin-1 beta

production by human corneal cells," Reg. Immunol. 2:136 (1989).

56. Kondo et al., "Interleukin-1 receptor antagonist suppresses contact hypersensitivity," J. Invest. Dermatol. 105:334 (1995).

57. Rosenbaum et al., "Activity of an interleukin 1 receptor antagonist in rabbit models of uveitis," Arch. Ophthalmol. 110:547 (1992).

58. Dinarello, "Interleukin-1 and interleukin-1 antagonism," Blood 77:1627 (1991).

While the present invention has been described in conjunction with a preferred embodiment, one of ordinary skill, after reading the foregoing specification, will be able to effect various changes, substitutions of equivalents, and other alterations to the compositions and methods set forth herein. It is therefore intended that the protection granted by Letters Patent hereon be limited only by the definitions contained in the appended claims and equivalents thereof.

CLAIMS

What is claimed is:

1. A method for prophylaxis or treatment of corneal transplant rejection comprising

providing a corneal transplant recipient patient; and

topically applying a therapeutic composition to an affected area of said patient, wherein said therapeutic composition comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for topical application.

2. A method for prophylaxis or treatment of an immunogenic inflammatory disease comprising

providing a patient suffering from or believed to be at risk from an immunogenic inflammatory disease of the eye; and

topically applying a therapeutic composition to an affected area of said patient, wherein said therapeutic composition comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for topical application.

3. A method for prophylaxis or treatment of an immunogenic inflammatory disease in an intraocular compartment comprising

providing a patient suffering from or believed to be at risk from an immunogenic inflammatory disease in an intraocular compartment; and

locally applying a therapeutic composition to an affected area of said intraocular compartment of said patient, wherein said therapeutic composition comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for local application.

4. A method for prophylaxis or treatment of corneal neovascularization comprising

providing a patient suffering from or believed to be at risk from corneal neovascularization; and

topically applying a therapeutic composition to an affected area of said patient, wherein said therapeutic composition comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for topical application.

5. The method of claim 2 wherein said patient is suffering from keratoconjunctivitis sicca, allergic conjunctivitis, corneal neovascularization, or immune or infectious keratitis states.

6. The method of claim 3 wherein said patient is suffering from uveitis or post-surgical inflammation.

7. The method of claim 3 wherein said applying step is by intraocular injection into the anterior chamber.

8. The method of claim 3 wherein said applying step is by intraocular irrigation at the time of surgery.

9. The method of claim 1, claim 2, claim 3 or claim 4 wherein said interleukin-1 antagonist in said therapeutic composition is an interleukin-1 receptor antagonist.

10. The method of claim 1, claim 2, claim 3 or claim 4 wherein said interleukin-1 antagonist in said therapeutic composition is IL-1ra.

11. The method of claim 1, claim 2, claim 3 or claim 4 wherein said carrier vehicle in said therapeutic composition comprises sodium hyaluronate.

12. A method for prophylaxis or treatment of corneal transplant rejection comprising

providing a corneal transplant recipient patient; and

topically applying a therapeutic composition to an affected area of said patient, wherein said therapeutic composition comprises a therapeutically effective amount of IL-1ra in association with a pharmaceutically acceptable carrier vehicle for topical application, said vehicle comprising sodium hyaluronate.

13. An article of manufacture comprising packaging material and a therapeutic composition contained within said packaging material, wherein the therapeutic composition is therapeutically effective for prophylaxis or treatment of corneal transplant rejection and wherein the packaging material comprises a label that indicates that the therapeutic composition can be used topically for prophylaxis or treatment of corneal transplant rejection, and

wherein said therapeutic composition comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for topical application.

14. An article of manufacture comprising packaging material and a therapeutic composition contained within said packaging material, wherein the therapeutic composition is therapeutically effective for prophylaxis or treatment of an immunogenic inflammatory disease of the eye and wherein the packaging material comprises a label that indicates that the therapeutic composition can be used topically for prophylaxis or treatment of an immunogenic inflammatory disease of the eye, and

wherein said therapeutic composition comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for topical application.

15. An article of manufacture comprising packaging material and a therapeutic composition contained within said packaging material, wherein the therapeutic composition is therapeutically effective for prophylaxis or treatment of an immunogenic inflammatory disease in an intraocular compartment and wherein the packaging material comprises a label that indicates that the therapeutic composition can be used locally for prophylaxis or treatment of an immunogenic inflammatory disease in an intraocular compartment of a patient, and

wherein said therapeutic composition comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for local application.

16. An article of manufacture comprising packaging material and a therapeutic composition contained within said packaging material, wherein the therapeutic composition is therapeutically effective for prophylaxis or treatment of corneal neovascularization and wherein the packaging material comprises a label that indicates that the therapeutic composition can be used topically for prophylaxis or treatment of corneal neovascularization, and

wherein said therapeutic composition comprises a therapeutically effective amount of an interleukin-1 antagonist in association with a pharmaceutically acceptable carrier vehicle for topical application.

17. The article of manufacture of claim 13, claim 14, claim 15 or claim 16 wherein, in said therapeutic composition, said interleukin-1 antagonist is an interleukin-1 receptor antagonist.

18. The article of manufacture of claim 13, claim 14, claim 15 or claim 16 wherein, in said therapeutic composition, said interleukin-1 antagonist is IL-1ra.

19. The article of manufacture of claim 13, claim 14, claim 15 or claim 16 wherein, in said therapeutic composition, said carrier vehicle comprises sodium hyaluronate.

20. A method for administering a therapeutic agent to an ocular surface of an eye of a patient comprising topically applying a therapeutic composition to the ocular surface of said patient, wherein said therapeutic composition comprises a therapeutically effective amount of a therapeutic agent in association with a pharmaceutically acceptable carrier vehicle for topical application, said carrier vehicle comprising a hyaluronate salt.

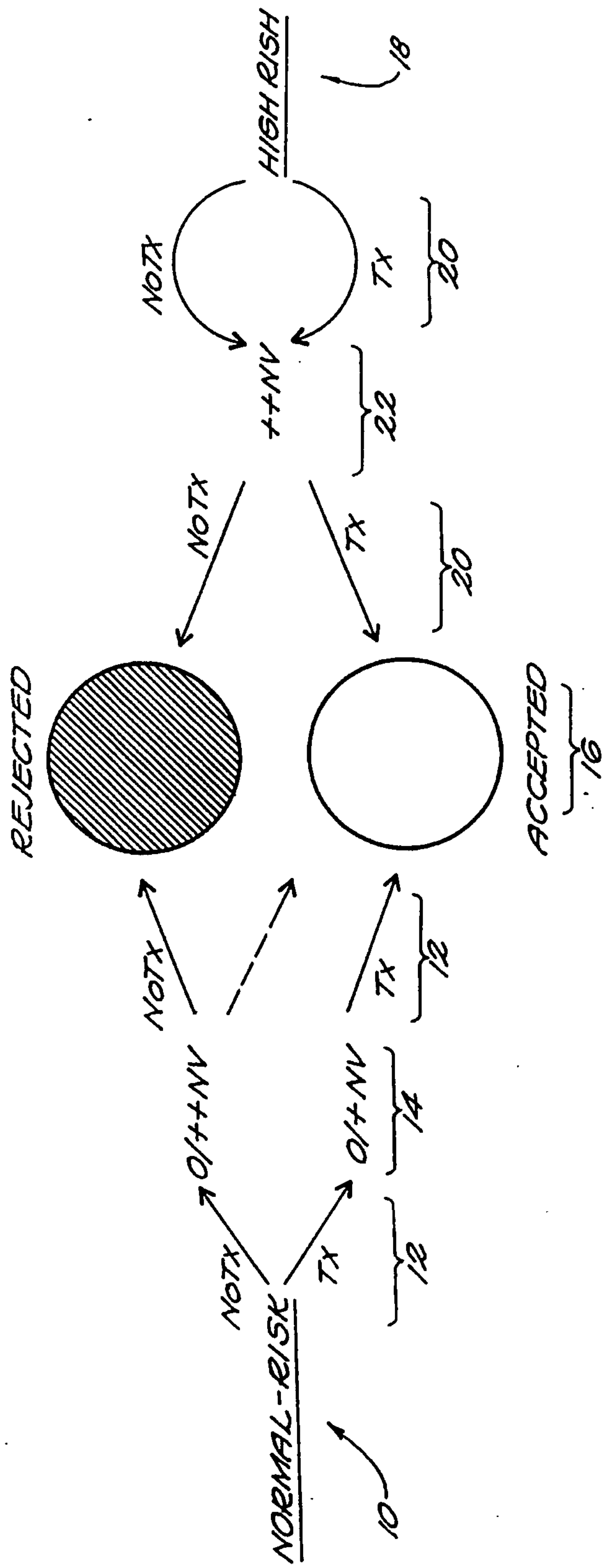

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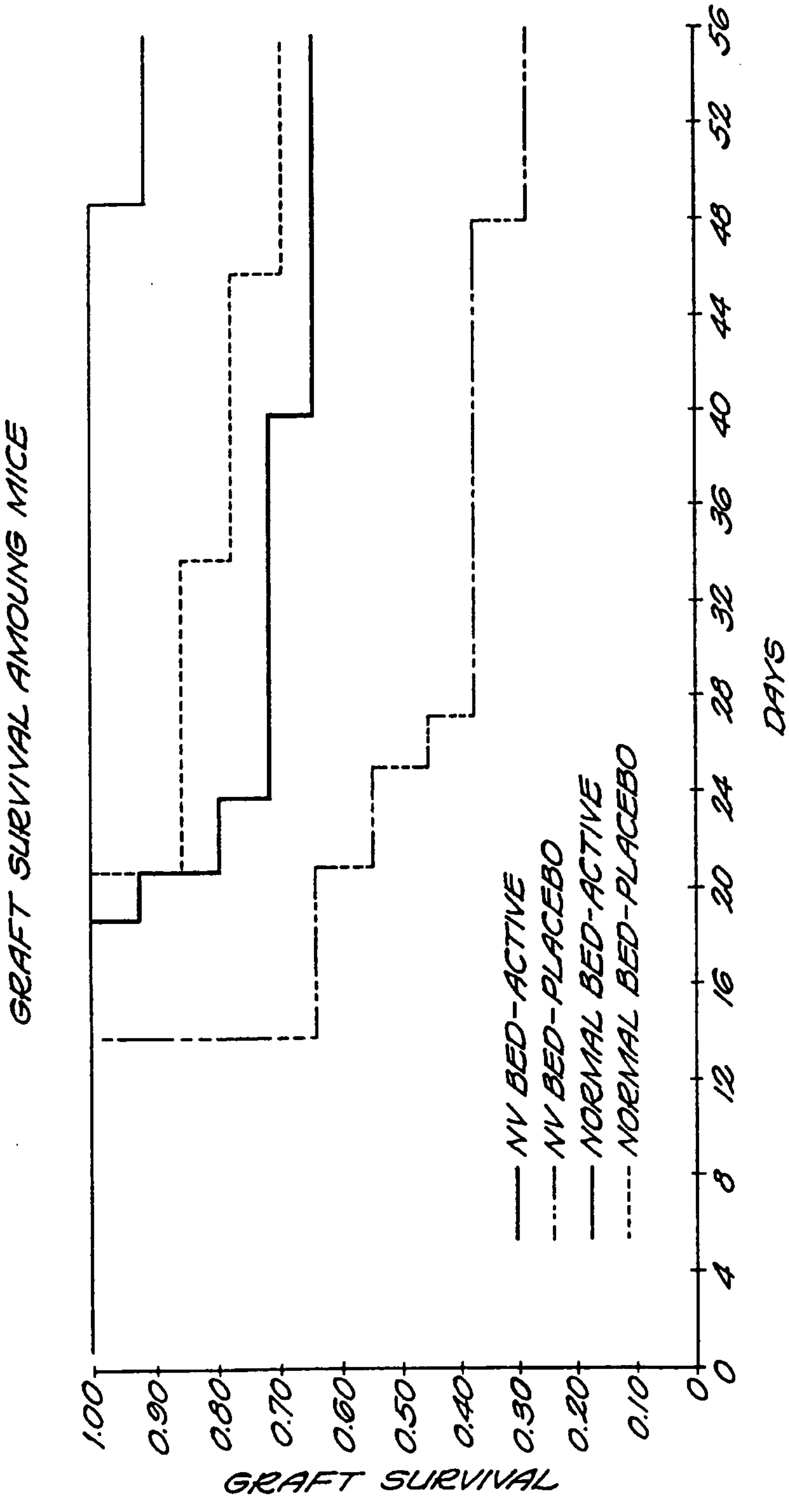
FIG. 1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NV BED-PLACEBO
NORMAL BED-ACTIVE
NORMAL BED-PLACEBO
GRAFT SURVIVAL
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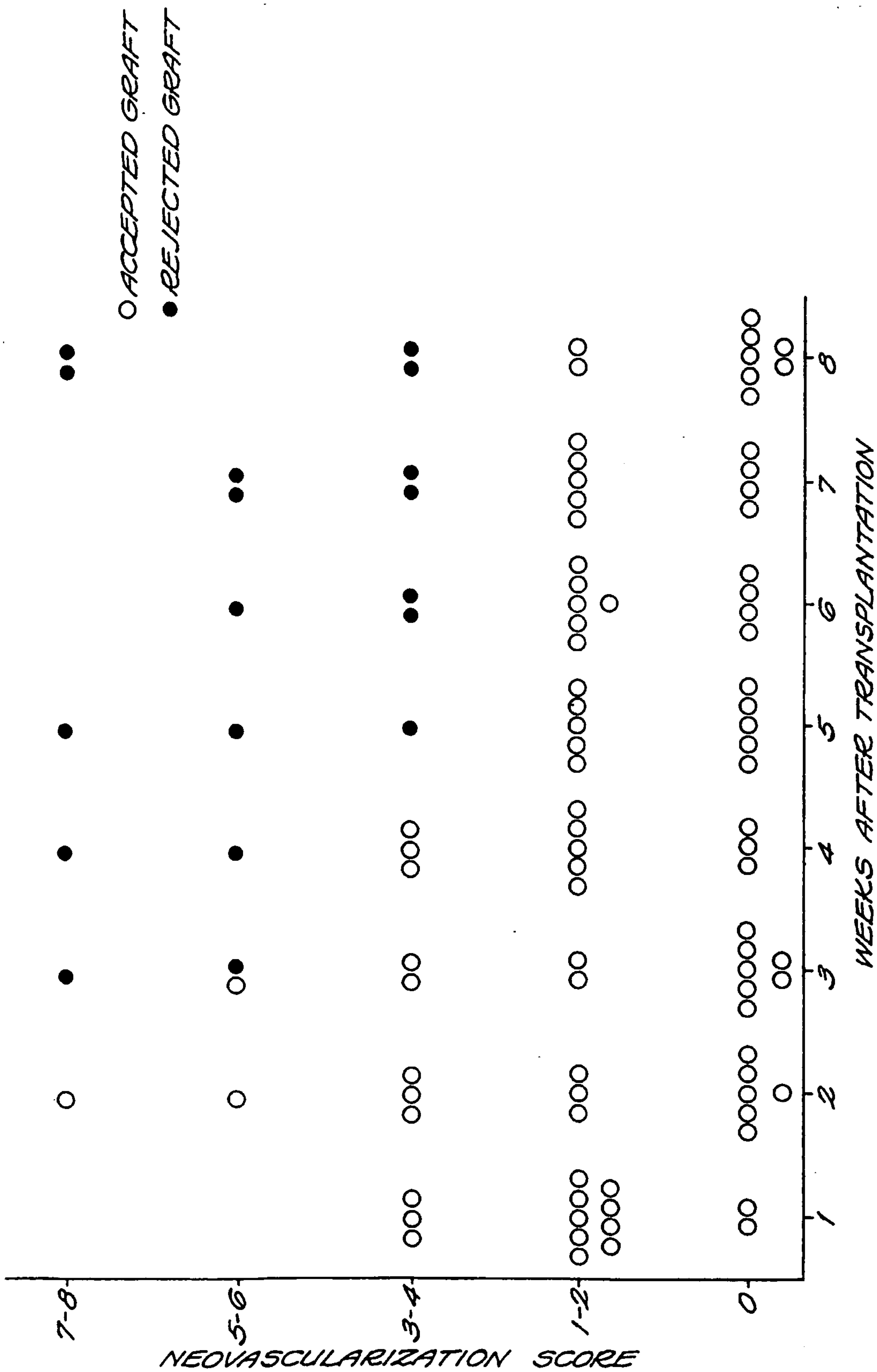
FIG. 2



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3-4
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FIG. 3A

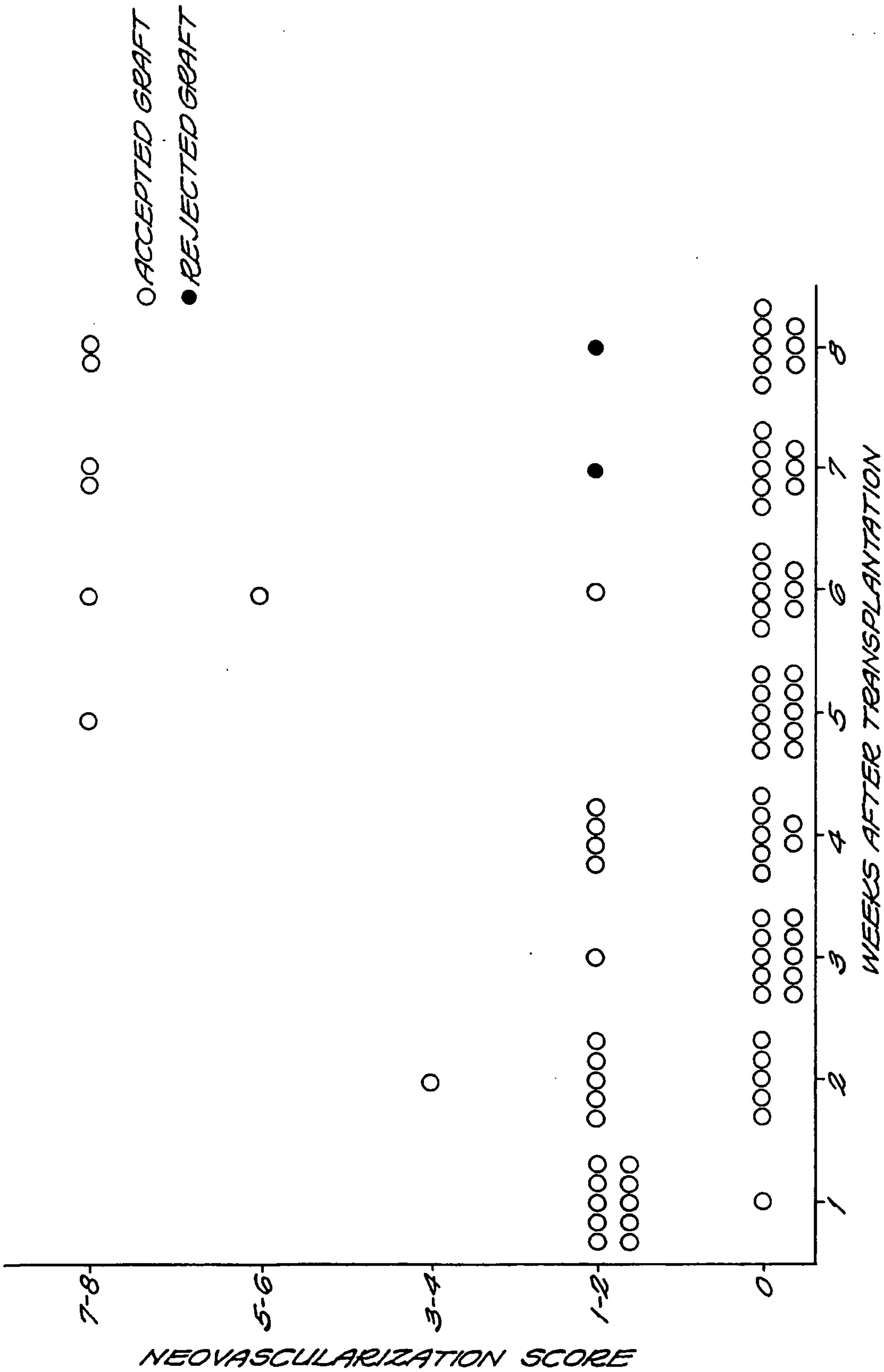

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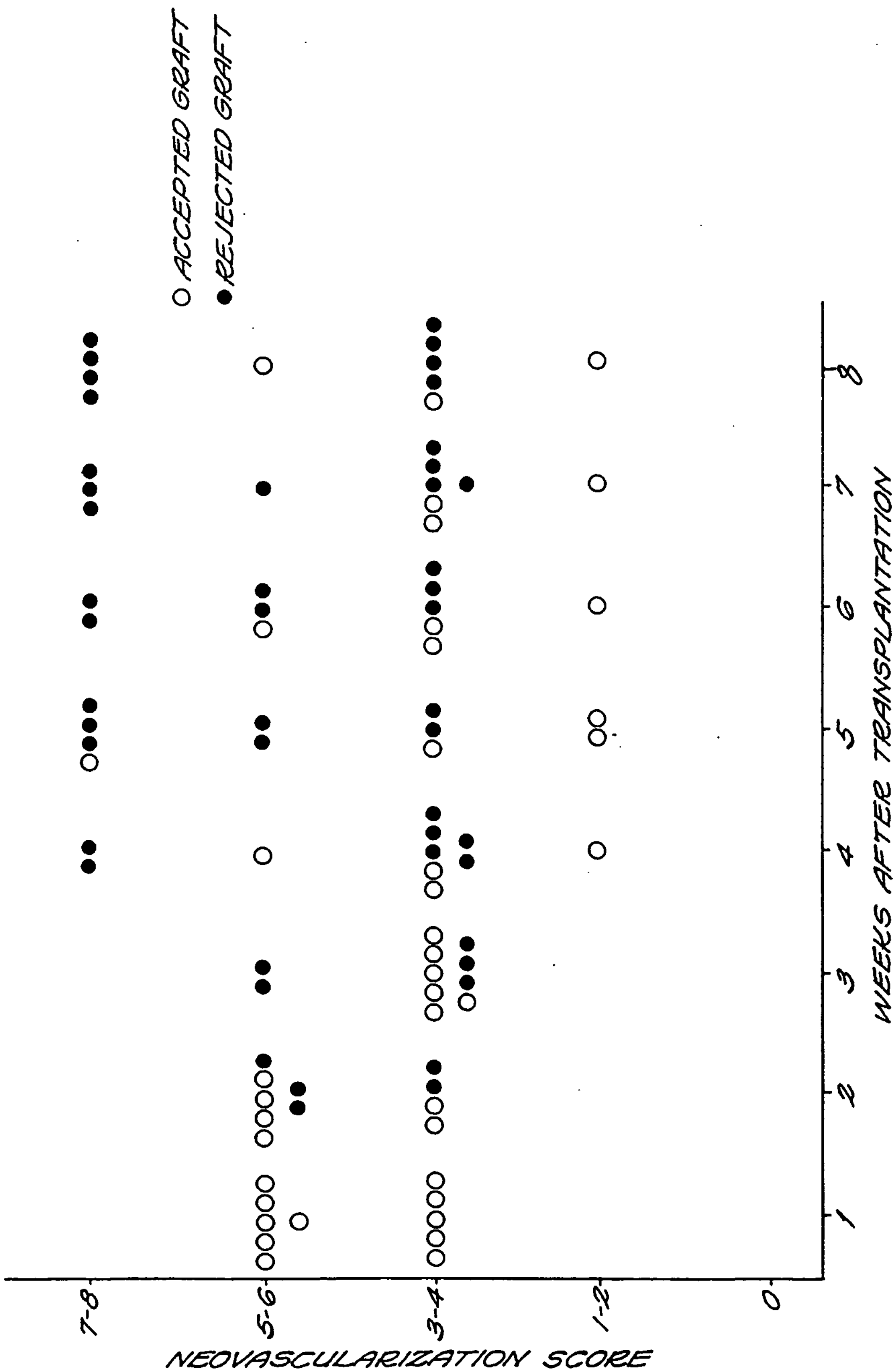
FIG. 3B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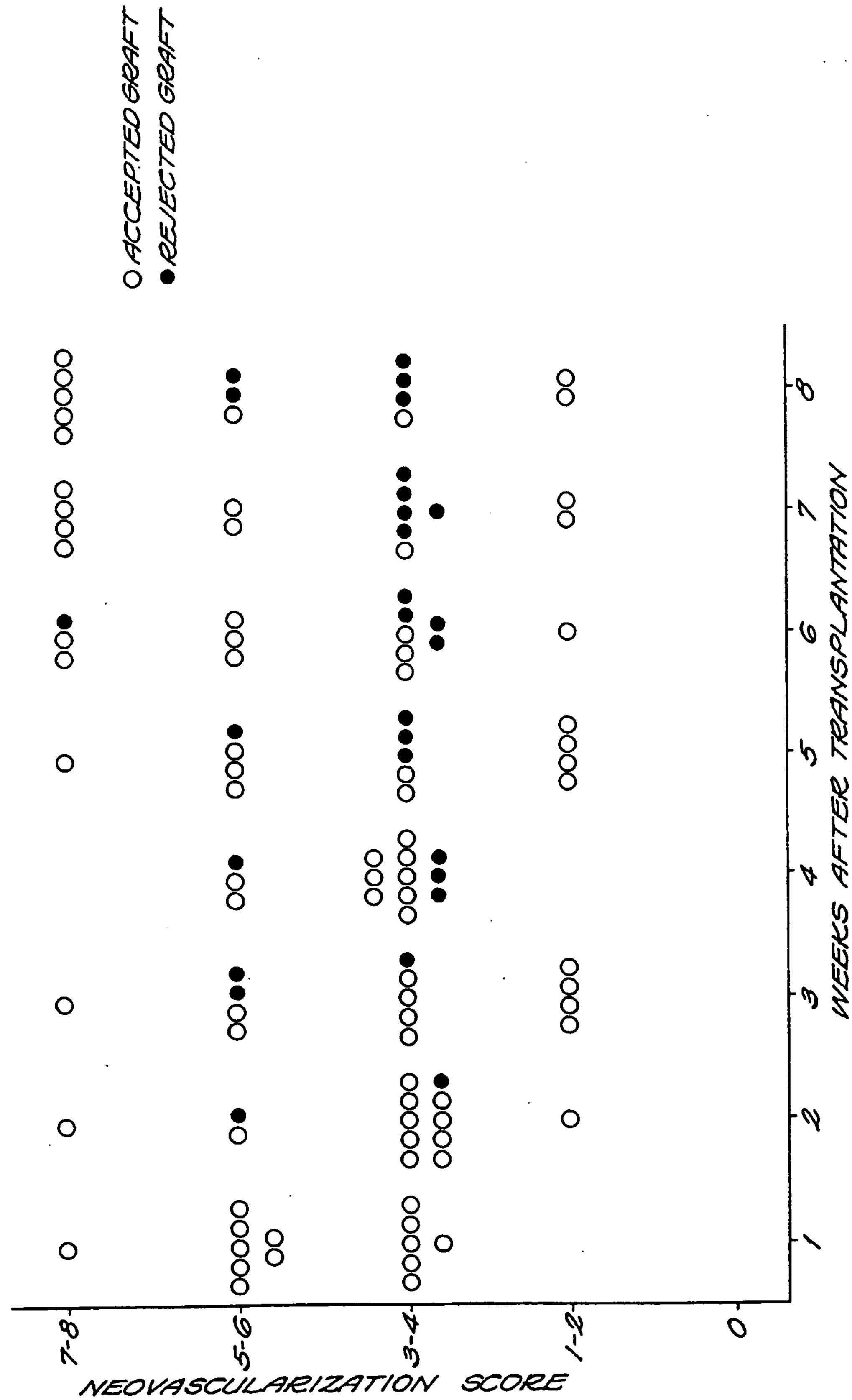
WEEKS AFTER TRANSPLANTATION

FIG. 4A



ACCEPTED GRAFT
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NEOVASCULARIZATION SCORE
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WEEKS AFTER TRANSPLANTATION
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FIG. 4B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/21393

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 09323 A (DOMPE) 28 March 1996 see the whole document ---	1-20
X	ROSENBAUM, JAMES T. ET AL: "Activity of an interleukin 1 receptor antagonist in rabbit models of uveitis" ARCH. OPHTHALMOL. (CHICAGO) (1992), 110(4), 547-9, XP002059494 see the whole document --- -/--	1-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
19 March 1998	08.04.98
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Moreau, J

Inte ional Application No
PCT/US 97/21393

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US TORRES P F ET AL: "Cytokine mRNA expression during experimental corneal allograft rejection." XP002059499 see abstract & EXPERIMENTAL EYE RESEARCH 63 (4). 1996. 453-461, ---	1-20
A	KENNEDY M C ET AL: "Novel production of interleukin - 1 receptor antagonist peptides in normal human cornea." JOURNAL OF CLINICAL INVESTIGATION 95 (1). 1995. 82-88, XP002059496 see the whole document ---	1-20
A	TORRES P ET AL: "Interleukin 1-beta and interleukin 1 receptor antagonist expression during experimental corneal graft rejection." ANNUAL MEETING OF THE INVESTIGATIVE OPHTHALMOLOGY AND VISUAL SCIENCE, FORT LAUDERDALE, FLORIDA, USA, MAY 14-19, 1995. INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE 36 (4). 1995. S1009, XP002059497 see the whole document ---	1-20
P,X	DANA M R ET AL: "Topical interleukin - 1 receptor antagonist (IL - 1ra) suppresses Langerhans cell activity and promotes immune privilege." ANNUAL MEETING OF THE ASSOCIATION FOR RESEARCH IN VISION AND OPHTHALMOLOGY, PARTS 1-2, FORT LAUDERDALE, FLORIDA, USA, MAY 11-16, 1997. INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE 38 (4 PART 1-2). 1997. S705, XP002059495 see the whole document ---	1-20
P,X	DANA M R ET AL: "Topical interleukin 1 receptor antagonist promotes corneal transplant survival." TRANSPLANTATION (BALTIMORE) 63 (10). 1997. 1501-1507, XP002059498 see the whole document -----	1-20

1

International application No.

PCT/US 97/ 21393

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-12,20
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-12,20
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/US 97/21393

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9609323 A	28-03-96	IT 1269989 B EP 0782584 A	16-04-97 09-07-97